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(54) Title: PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

(57) Abstract

The present invention includes a method for preventing or treating the toxic effects of a superantigen. A subject is treated with a molecule which interacts with specific Vβ elements of T cells in a manner similar to that of a native superantigen. The molecules of the present invention are mutated or modified superantigens which elicit antibody production without inducing T cell proliferation.

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PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

FIELD OF THE INVENTION

5   This invention relates to methods for preventing
and treating antigen-mediated and antigen-initiated
diseases. Specifically, it relates to providing
protection against superantigen pathogens by
administration of molecules which are modified or
10   mutated superantigens which elicit a antibody response
against the superantigen without having the
pathological effect of the superantigen. The molecules
of this invention may also interact with the $V\beta$
elements of T cell receptors in a way which leads to
15   modifications in the way T cells respond to an antigen.

BACKGROUND OF THE INVENTION

         The vertebrate immune system evolved to protect
vertebrates from infection by microorganisms and large
20   parasites. The immune system responds to antigens in
one of two ways: (1) humoral antibody responses,
mediated through B cells, involving the production of
protein antibodies which circulate in the bloodstream
and bind specifically to the foreign antigen which
25   induced them. The binding of the antibody to the
antigen makes it easier for phagocytic cells to ingest
the antigen and often activates a system of blood
proteins, collectively called complement, that helps
destroy the antigen; and (2) cell-mediated immune
30   responses, mediated through T cells, involving the
production of specialized cells that react mainly with
foreign antigens on the surface of host cells, either
killing the host cell if the antigen is an infecting
virus or inducing other host cells, such as
35   macrophages, to destroy the antigen (Molecular Biology
of the Cell (1983), B. Alberts et al. (eds), chapter
17, pp. 952).

         The production of antibodies requires a number of
preceding events to occur which lead to stimulation of

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B cells producing the antibodies. One of the key
events involved in the processes leading to antibody
production is that of antigen recognition. Antigen
recognition requires the participation of thymus (T)
5   cells.

T cells have antigen-specific receptors on their
surfaces, termed T cell antigen receptors (TCR).
Before T cells can recognize protein antigens, the
antigens must be presented on the surface of antigen-
10   presenting cells. The antigens must first be processed
by macrophages or other antigen presenting cells.
These cells essentially swallow antigens and chop them
into peptides which are displayed at the cell surface
in combination with major histocompability complex
15   (MHC) molecules.

The major histocompatibility antigens are a family
of antigens encoded by a complex of genes called the
major histocompatibility complex. MHC antigens are
expressed on the cells of all higher vertebrates. In
20   man they are called HLA antigens (human-leucocyte-
associated antigens) because they were first
demonstrated on leucocytes. There are two principal
classes of MHC molecules, class I and class II, each
comprising a set of cell-surface glycoproteins. The
25   two classes of MHC antigens stimulate different
subpopulations of T cells. MHC class II molecules are
involved in most responses to pathogens. In contrast,
MHC class I molecules are involved when the pathogen is
a virus or when a malignant cell is involved. When MHC
30   class I is involved, antibody stimulation does not
result; rather, the interaction of MHC class I
processed antigen and T-cell leads to lysis of cells
infected with the pathogen.

The processed antigen peptide fits in a cleft on
35   an MHC molecule. Once an antigen is displayed, the few
T cells in the body that bear receptors for that
particular peptide bind that complex. Most T cells

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recognize antigens on the surface of cells only in
association with self-MHC glycoproteins expressed on
the same cell surface.

The ability of the T cell to complex with the
processed antigen and MHC complex is dependent on the T
cell receptor (TCR). The TCR consists of two protein
chains, α and β. Each chain contains a constant and a
variable domain. The variable domains are encoded in
two (α) or three (β) different gene segments (variable
(V), diversity (D), joining (J)) (Siu et al. (1984)
Cell 37:393; Yanagi et al. (1985) Proc. Natl. Acad.
Sci. USA 82:3430). In each T cell, the combination of
V, D, and J domains of both the α and β chains
participates in antigen recognition in a manner which
is uniquely characteristic of that T cell and defines a
unique binding site. See, Marrack et al. (1988)
Immunol. Today 9:308; Toyonaga et al. (1987) Ann. Rev.
Immunol. 5:585; Davis (1985) Ann. Rev. Immunol. 4:529;
Hendrick et al. (1982) Cell 30:141; Babbitt et al.
(1985) Nature 317:359; Buus et al. (1987) Science
235:1353; Townsend et al. (1986) Cell 44:959; Bjorkman
et al. (1987) Nature 329:506). Generally, both the α
and β chains are involved in recognition of the ligand
formed by processed antigen and MHC.

When T cells are stimulated by an antigen, they
divide and differentiate into activated effector cells
that are responsible for various cell-mediated immune
reactions. At least three different reactions are
carried out by T cells: (1) cytotoxic T cells
specifically kill foreign or virus-infected vertebrate
cells; (2) helper T cells help B lymphocytes; and (3)
suppressor T cells supress the responses of specific
cells.

Recently, it has been shown that a novel class of
antigens, termed "superantigens", are able to directly
stimulate T cells by binding to a particular Vβ
element, that is, the variable domain of the β chain of

the TCR (Kappler et al. (1987) Cell 49:263; Kappler et al. (1987) Cell 49:273; MacDonald et al. (1988) Nature 332:40; Pullen et al. (1988) Nature 335:796; Kappler et al. (1988) Nature 332:35; Abe et al. (1988) J. Immunol.

5   140:4132; White et al. (1989) Cell 56:27; Janeway et al. (1989) Immunol. Rev. 107:61; Berkoff et al. (1988) J. Immunol. 139:3189; Kappler et al. (1989) Science 244:811). Unlike recognition of conventional peptide antigens, the other components of the T cell receptor

10   (i.e., Dβ, Jβ, Vα, Jα) appear to play little role in the superantigen binding. Superantigens, while generally stimulatory to T cells, appear to interact specifically with particular Vβ elements present on the stimulated T cell. Since the relative number of Vβ

15   genes is limited, many T cells within an individual will bear a particular Vβ element, and a given superantigen is therefore capable of interacting with a large fraction of the T cell repertoire. Thus, depending on the frequency of the responding Vβ

20   population(s), 5-30% of the entire T cell repertoire could be stimulated by a superantigen, whereas the responding frequency to a conventional antigen is usually much less than 1 in 1,000. Although superantigens interact with class II MHC molecules,

25   they appear to act as intact proteins rather than as peptides, that is, they do not appear to bind within the conventional peptide binding groove. Instead, they seem to interact with amino acid residues that are on the outer walls of the binding cleft. Known

30   superantigens and references to their sequences and structures are listed in Table I.

     Two distinct classes of superantigen have been described. The first was noted nearly 20 years ago, when Festenstein showed marked responses in mixed

35   lymphocyte reactions between certain MHC identical strains. The stimulating antigens were called minor lymphocyte stimulating (Mls) antigens (Festenstein

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(1973) Transplant Rev. 15:62) to differentiate them
from MHC antigens. These superantigens are encoded by
endogenous retroviral genes (Palmer (1991) Curr. Bio.
1:74). The presence of these genes in the mouse leads
to a marked deletion of responding T cells, creating
potentially large holes in the animal's T cell receptor
repertoire (Pullen et al. (1988) supra). The second
set of superantigen is represented by a growing list of
bacterial and viral proteins, capable of producing a
variety of pathological effects after injection
(Marrack & Kappler (1990) Science 248:705).

Staphylococcus aureus (S. aureus), a common human
pathogen, produces several enterotoxins, designated as
SEA (staphylococcal enterotoxin A) through SEE, which
can be responsible for food poisoning and occasionally
shock in humans (Marrack & Kappler (1990) supra;
Bohach et al. (1990) Crit. Rev. Microbio. 117:251).
Some S. aureus isolates also produce toxic shock
syndrome toxin-1 (TSST-1), which has been implicated in
the majority of cases of human toxic shock syndrome as
well as the related exfoliative toxins (ExTF), which
are associated with the scalded skin syndrome.
Streptococcus pyrogenes, or group A streptococcus,
another common human pathogen of the skin and pharynx,
also produces toxins with superantigenic properties
(Abe et al. (1991) J. Immun. 146:3747). These have
been designated streptococcal erythrogenic toxins A-C
(SPEA-C).

The amino acid sequence of the S. aureus toxins
exhibit some homology, but also exhibit marked
differences (See, Bentley et al. (1988) J. Bacteriol.
170:34; Jones et al. (1986) J. Bacteriol. 166:29; Lee
et al. (1988) J. Bacteriol. 170:2954; Blomster-Hautamaa
et al. (1986) J. Biol. Chem. 261:15783). S. aureus
has the ability to stimulate powerful T cell
proliferation responses in the presence of mouse cells
bearing MHC class II type molecules (Carlson et al.

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(1988) J. Immunol. 140:2848; White et al. (1989) Cell
56:27). The S. aureus proteins selectively stimulate
murine cells bearing particular Vβ elements.

5   The binding of toxins to class II MHC molecules is
a prerequisite for T cell recognition, but the process
is much more permissive for superantigens than that
seen with conventional antigens. While peptide
antigens are very dependent on allelic MHC residues for
binding, the superantigens bind to a wide variety of
10   allelic and isotypic forms of MHC class II molecules
(See, Hermann et al. (1989) Eur. J. Immunol. 19:2171;
Herman et al. (1990) J. Exp. Med. 172:709; Scholl et
al. (1990) J. Immunol. 144:226; Molleck et al. (1991)
J. Immunol. 146:463). While T cells rarely recognize
15   peptide antigens bound to self-MHC (allo-MHC)
molecules, individual T cell clones can respond to
toxins bound not only to various allelic forms of MHC,
but also to different class II isotypes and even to
xenogenic class II molecules. Such observations
20   suggest that superantigens bind at a relatively
conserved site outside the allelically hypervariable
groove thought to bind conventional peptide antigens.

      Superantigens may contribute to autoimmune
diseases, in which components of the immune system
25   attack normal tissue. The process of deletion of T
cells responsive to self, potentially harmful self-
reactive T cells, is called tolerance or negative
selection (Kappler et al. (1987) Cell 49:273; Kapper et
al. (1988) Nature 332:35; MacDonald et al. (1988)
30   Nature 332:40; Finkel et al. (1989) Cell 58:1047). The
immune system normally deletes self-reactive T cells,
but occasionally a few appear to escape the
surveillance mechanism. It has been suggested that the
ability of superantigens to arouse 20 percent of a
35   person's T cell repertoire could lead to undesirable
replication of the few circulating T cells that
recognize self (Johnson et al. (1992) Scientific

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American 266:92). T cells bearing certain Vβ types
have been implicated in various autoimmune conditions,
including arthritis and multiple sclerosis. These
findings imply that the destructive cells might be
activated by a superantigen that binds to the
identified Vβ types (Johnson et al. (1992) supra).

Autoimmune diseases are a result of a failure of
the immune system to avoid recognition of self. The
attack by the immune system of host cells can result in
a large number of disorders, including such neural
diseases as multiple sclerosis and myasthenia gravis,
diseases of the joints, such as rheumatoid arthritis,
attacks on nucleic acids, as observed with systemic
lupus erythematosus, and such other diseases associated
with various organs, as psoriasis, juvenile onset
diabetes, Sjögren's disease, and thyroid disease.
These diseases can have a variety of symptoms, which
can vary from minor and irritating to life-threatening.
For example, rheumatoid arthritis (RA) is a chronic,
recurrent inflammatory disease primarily involving
joints, and affects 1-3% of North Americans, with a
female to male ration of 3:1. Severe RA patients tend
to exhibit extra-articular manisfestations including
vasculitis, muscle atrophy, subcutaneous nodules,
lymphadenopathy, splenomegaly, and leukopenia. It is
estimated that about 15% of RA patients become
completely incapacitated.

Several lines of evidence suggest that T cells
specific for self-antigens may play a critical role in
the initiation of autoimmune diseases. In the case of
RA, the linkage of the disease to the DR4 and DR1
alleles of the class II genes of MHC and the findings
that sometimes oligoclonal, activated CD4$^+$ T cells in
synovial fluid and tissue of affected joints (Stastny
et al. (1976) Engl. J. Med. 298:869; Gibofsky et al.
(1978) J. Exp. Med. 148:1728; McMichael et al. (1977)
Arth. Rheum. 20:1037; Schiff et al. (1982) Ann. Rheum.

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Dis. 41:403; Duquestoy et al. (1984) Hum. Immunol.
10:165; Legrand et al. (1984) Am. J. Hum. Genet.
36:690; Gregerse et al. (1987) Arth. Rheum. 32:15;
Burmester et al. (1981) Arth. Rheum. 24:1370; Fox et
al. (1982) J. Immunol. 128:351; Hemler et al. (1986) J.
Clin. Invest. 78:696; Stamenkoic et al. (1988) Proc.
Natl. Acad. Sci. USA 85:1179) suggest the involvement
of CD4⁺, αβTCR-bearing, class II-restricted T cells in
the disease. This view is supported by the finding
that partial elimination or inhibition of T cells by a
variety of techniques can lead to an amelioration of
disease in certain patients (Paulus et al. (1977) Arth.
Rheum. 20:1249; Karsh et al. (1979) Arth. Rheum.
22:1055; Kotzin et al. (1989) N. Eng. J. Med. 305:976;
Herzog et al. (1987) Lancet ii:1461; Yocum et al.
(1989) Ann. Int. Med. 109:863).

U. S. patent application Serial No. 07/732,114,
herein specifically incorporated by reference,
establishes that specific Vβ elements may be used to
diagnose for an autoimmune disease, specifically the
presence of a higher percentage of Vβ14⁺ T cells in
synovial fluid may be used to diagnose RA.

Many investigative efforts have focused on
developing methods for the treatment of autoimmune
diseases. For example, European Patent Publication 340
109, entitled Anti-T-cell receptor determinants as
autoimmuune disease treatment, and U.S. Patent No.
4,550,086, issued October 29, 1985 to Reinherz et al.,
entitled Monoclonal antibodies that recognize human T
cells, describe a method of detecting a particular
sequence of the variable region gene of T cell
receptors associated with a particular disease and
treating the disease with antibodies to that sequence.
U.S. Patent No. 4,886,743, issued December 12, 1989 to
Hood et al., entitled: Diagnostic reagents based on
unqiue sequences within the variable region of the T
cell receptor and uses thereof, describes a method of

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diagnosis diseases based on the presence of T cells
with a unique sequence in the $V\beta$ region associated with
a specific disease. PCT Patent Application Publication
WO 90/06758 describes a method for detecting specific

5       $V\beta$ regions associated with RA, specifically, $V\beta3$, $V\beta9$,
and $V\beta10$, and for the treatment of RA with monoclonal
antibodies which recognize $V\beta3$, $V\beta9$, and $V\beta10$.

Immunity

10   An animal that has never been exposed to a
pathogen has no specific defenses against it. However,
the animal can be immunized against the pathogen by
injecting it with a non-virulent form of the pathogen
with a similar chemical structure as the pathogen but

15   without the ability to cause the pathological effect.
The animal will produce antibodies specific against the
non-virulent form of the pathogen, and these antibodies
can protect the animal against attack from the virulent
pathogen.

20

BRIEF SUMMARY OF THE INVENTION
            The present invention includes a method for
preventing the toxic effects of a superantigen by
treatment with a molecule, wherein said molecule

25   elicits antibody production without inducing T cell
activation.
            The present invention also includes molecules
consisting of mutated or modified derivatives of
superantigens.

30   The present invention further includes a method of
modifying T cell response elicited by an antigen
comprising administering a molecule which interacts
with either the $V\beta$ element alone or both the α and β
chains of T cell receptors (TCR).

35   The molecules of this invention can function by
leading to deletion or inactivation/desensitization of

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at least one or more subpopulations of T cells which
present a particular Vβ element.

To prevent the _in vivo_ toxic effect of
superantigen requires an exact understanding of how
their effect is achieved. Prior to this invention,
while it was known how superantigen interact with T
cells, the manner in which a subject animal developed a
pathological condition and whether a pathological
condition would develop was not well understood.
Various observations suggested that any of a number of
mechanisms could be the cause of the toxicity.

It has now been found that the pathological
condition mediated or initiated by a superantigen can
be prevented or treated by administration of the mutant
superantigen molecules of the present invention.
Administration of the mutant toxins of the present
invention may cause antibody production against the
mutant molecule. Some of these antibodies also react
with the normal non-mutated toxin. Therefore, when the
immunized individual is confronted with the normal
toxin, these cross-reactive antibodies react with the
normal toxin and inhibit its toxic activity.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is a schematic ribbon drawing of the
three dimensional structure of SEB. Region 1 (residues
9-23), region 2 (residues 40-53), and region 3
(residues 60-61) are differentiated by shading. Sites
identified as involved in MHC or TCR binding are shown.
Residues identified by mutational analysis as important
to MHC and/or TCR binding are indicated.

FIGURE 2a shows the SDS-PAGE analysis of 2 ug of
recombinant SEB purified from _E. coli_ and wild-type SEB
purified from _S. aureus_ cultures. Molecular mass
markers (in kD): β-phosphorylase, 94; bovine albumin,

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69; ovalbumin, 45; carboxylase, 30; soybean trypsin
inhibitor, 21; lysozyme, 14. FIGURE 2b shows the SDS-
PAGE analysis of SEB binding to DR on LG2 cells.
Molecular mass markers (in kD): bovine albumin, 69;
ovalbumin, 45; chymotrypsinogen, 27; soybean trypsin
inhibitor, 21; myoglobin, 17; lysozyme, 14.

FIGURE 3 shows the sequences of the SEB mutants.
Dashed lines indicate identity to unmutated SEB. The
positions of the oligonucleotides used to generate the
SEB mutants are also shown.

FIGURE 4 shows the binding of SEB and SEB mutants
to DR1-bearing lymphoblastoid line LG2 cells.

FIGURE 5 shows stimulation of T cell hybridomas by
region 1 SEB mutants. Preparations of purified SEB or
the region 1 mutants were tested for their ability to
stimulate a collection of T cell hybridomas bearing of
the the Vβ elements known to recognize SEB: KS-20.15
(Vβ7), KS-6.1 (Vβ8.2), KS-47.1 (Vβ8.3), K16-57 (Vβ8.1).

FIGURE 6 shows stimulation of T cell hybridomas by
region 2 SEB mutants. Preparations of purified SEB
were tested as in Figure 5.

FIGURE 7 shows stimulation of T cell hybridomas by
region 3 SEB mutants. Preparations of purified SEB
were tested as in Figure 5.

FIGURE 8 shows the effects of SEB and its mutants
in vivo. Groups of three mice were weighed and then
given balanced salt solution (BSS) containing nothing,
50 ug (left), or 100 ug (right) of recombinant SEB or
the mutant SEBs BR-257 or BR-358.

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FIGURE 9 shows the protective effect of mutant toxins against challenge with SEB. Mice received doses of either saline or BR-257 three months prior to challenge with wild-type SEB.

5

DETAILED DESCRIPTION OF THE INVENTION

The molecules of the present invention may be effective in different ways in preventing or treating antigen-mediated or initiated diseases. Some of the
10 different ways in which the molecules of the present invention may be effective include modification of the T cell response and production of antibodies which provide protection against pathogens. Specifically, this invention presents a method of preventing or
15 treating superantigen-mediated or superantigen initiated diseases. The method of this invention generally involves preparing mutated superantigen molecules by methods known in the art and described herein, identifying antigen mutants unable to bind
20 either MHC or TCR, and testing for ability to protect against exposure to the non-mutated superantigen.

The present invention describes the feasibility of the above-outlined approach in achieving protection against a known superantigen. Mutants of recombinant
25 Staphylococcal enterotoxin B (SEB) were prepared and purified as described in Examples 1-4 below. SEB mutants unable to bind MHC molecules or TCR were selected by examining the binding of mutant SEB molecules to HLA-DR1 homozygous lymphoblastoid line LG2
30 cells and stimulation of T cell hybridomas bearing different Vβ elements. The SEB mutant BR-257, which bound LG2 cells in a manner indistinguishable from that of non-mutated SEB and did not stimulate T cell hybridomas, injected into experimental animals 3 months
35 prior to exposure to SEB provided complete protection against the toxic effects of SEB. Similar results were obtained in primates.

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Although the present disclosure describes production of mutated SEB molecules able to protect animals against subsequent challenge with SEB, the methods of the present invention are equally applicable to other superantigens.

The ability to use the occurrence of specific $V\beta$ elements to diagnose autoimmune diseases, as discussed in detail above, may be combined with the present invention as a method of detecting and treating autoimmune diseases mediated by superantigens. The existence of a superantigen-mediated disease may be determined by a "footprint" analysis, e.g., by determining if there is an alteration in $V\beta$ elements in a disease state. The finding of alterations in $V\beta$ elements, such as the increase in $V\beta14^+$ T cells in synovial fluid in RA, suggests the presence of a superantigen-mediated disease. Techniques known to the art may then be applied in order to isolate and identify the implicated superantigen. The $V\beta$ footprint may be compared against that of a known superantigen for possible implication of that superantigen in initiation or proliferation of the disease. There may a search for genes coding for a superantigen when a virus or bacterial infection is associated with the initiation of the disease. Once a superantigen is identified or isolated, the method of the present invention may be applied to produce a mutant superantigen molecule capable of conferring protection against exposure to the superantigen.

Various terms are used in this specification, for which it may be helpful to have definitions. These are provided herein, and should be borne in mind when these terms are used in the following examples.

As described above, the key event in an immune response is the interaction of MHC molecules with antigens to form a complex presented to T cells. Generally, the T cell response is quite specific, with

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only very limited subpopulations of T cells responding
to specific complexes of antigen and MHC molecules.
The response generally requires interaction of most or
all of the components of the T cell receptor. In

5   certain circumstances, however, the presented antigen
need only interact with the Vβ element of the receptor,
all other components are essentially irrelevant. This
means that the antigen can, and does, react with a much
greater array of T cells than is normally the case.

10   The molecules of this invention may interact with
the Vβ elements of T cell receptors in a way which
leads to modifications in the way T cells respond to a
superantigen. "Modifying T cell responsiveness" means
that the mutant molecules are able to change the manner

15 in which the subject's T cells respond when provoked by
the administered molecule, or to an antigen
administered concurrently, previously, or afterward.
For example, it is believed that early in the
development of T cells, certain subpopulations interact

20 with presented antigens and are deleted. The molecules
of this invention can function in this manner, i.e., by
leading to deletion or inactivation/desensitization of
at least one or more subpopulations of T cells which
present a particular Vβ element.

25   In a particular embodiment of the present
invention, the molecules modify the T cell response
without changing the B cell response that would
normally occur in the subject under consideration.
This type of material is useful, for example, for

30 providing passive immunity to a subject, or serving as
a vaccine. When superantigen derivatives are used,
these derivatives are no longer superantigenic, as they
will not provoke a restricted T cell response, but will
still serve as antigens in that they generate a B cell

35 response. The superantigen derivatives of the present
invention are able to elicit normal antibody production
against the superantigen protein.

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The molecules of the present invention may also be
seen as competitors for other antigens. If the
molecules described herein interact with MHC elements
otherwise required for generation of a full scale
5   response to an antigen or superantigen, they may
prevent or reduce the extent of that response.

The molecules of the present invention may also be
viewed as "enhancers" in some instances, where an
individual's T cell responsiveness is impaired or
10   weakened by any of a number of causes. Via
administration of the molecules encompassed by the
present invention, the T cell populations of the
individual can be greatly expanded.

The term "modifying T cell responsiveness" as used
15   herein is always relative to a second element (e.g., an
antigen), and always refers in particular to
responsiveness of T cells presenting a particular Vβ
element as part of their T cell receptors, other
components of the receptors being essentially
20   irrelevant.

The molecules of the present invention contain, at
least, an amino acid sequence of sufficient size to
bind to an MHC molecule. The rest of the molecule may
consist of amino acids, or may contain carbohydrate or
25   lipid structures.

"Reducing responsiveness" is construed to also
include deleting the portion of T cells expressing a
particular Vβ element.

"Superantigen derivative" as used herein refers to
30   a molecule whose structure, at the least, contains an
amino acid sequence substantially identical to an amino
acid sequence presented by a superantigen or portions
of a superantigen required for binding to either the
MHC or the T cell.

35   "Modified" superantigen derivative (or fragment),
differs from "mutated" superantigen derivative (or
fragment). The term "modified superantigen" is defined

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to refer to molecules which contain an amino acid
sequence identical to an amino acid sequence of
superantigen, but contain modifications not found in
the superantigen molecule itself. For example, if a
5   superantigen contains amino acids 1-250, a "modified"
superantigen derivative may contain a sequence
identical to amino acids 50-75, positioned in between
stretches of amino acids not found in the native
superantigen molecule. Additional modifications may
10   include, for example, differing or absent glycosylation
patterns, or glycosylation where there normally is
none.
        "Mutated" superantigen refer to structures where
the actual amino acid sequence of the mutation has been
15   altered relative to the native form of the molecule.
For example, if a superantigen contains amino acids 1-
250, a mutated superantigen may include amino acids 50-
68 and 72-75 which are identical to the corresponding
native sequence, but differ in amino acids 69-71. The
20   difference may be one of "substitution" where different
amino acids are used, "addition" where more amino acids
are included so that the sequence is longer than the
native form, or "deletion" where the amino acids are
missing.
25   "Vaccine" refers to a formulation when
administered to a subject provokes the same type of
response typical of vaccines in general, e.g., active
immunological prophylaxis. The vaccine may contain
adjuvant, or other materials.
30   It is known that the class of molecules known as
superantigens interact with particular $V\beta$ regions of T
cell receptors, leading to massive proliferation of
particular T cell subpopulations. This interaction,
which assumes prior interaction between an MHC molecule
35   and the superantigen, is almost completely independent
of any other region of the T cell receptor.

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In connection with the interaction of MHC and peptide, it must be noted that MHC molecules are available in a variety of "phenotypes", and different phenotypes are specific for various presented peptides and antigens. For example, HLA-DR is known to be associated with presentation of SEB. Thus, different MHC phenotypes will be of value for different antigens, but determination of HLA phenotype and correlation to presentation of a particular antigen or antigen family is well within the skill of the artisan in this field.

Thus, this invention involves the modification of the T cell response via administration to a subject of a molecule which interacts with both an MHC molecule and at least one Vβ element on T cell receptors. This interaction may affect the T cell response in any number of ways. Perhaps the most elementary manner of affecting the response is one where a molecule interacts with the MHC molecule, preventing the binding of other molecules to the MHC. If the competing molecule has been modified or does not naturally provoke proliferation of T cells, then there will be a lessening or elimination of the response because molecules such as normal antigens or superantigens cannot form the requisite complex with the MHC to generate a T cell proliferative response.

Another manner of modifying the T cell response is via "desensitizing", "inactivating", or "anergizing" the T cells. This mechanism involves interaction of MHC molecule, antigen, and T cell receptor, with subsequent down regulation or inactivation of the T cells. This mechanism is more common in mature subjects than the deletion phenomenon, which occurs in fetal subjects. The latter phenomenon is one where via interaction of the three units, various subpopulations of T cells are in fact removed from the organism.

The modification of the T cell response can also involve stimulation of T cell subpopulations.

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Knowledge of the mechanisms described herein permits
the artisan to administer to a subject a material which
interacts with the MHC and a particular subpopulation
of T cells, where proliferation of the T cell

5   subpopulation results. This approach is particularly
desirable in the treatment of conditions where a
particular Vβ subpopulation or subpopulations are
associated with a pathological condition, such as an
autoimmune disease.

10   It should be understood that an immune response,
when fully considered, includes both a B cell and a T
cell response. One aspect of the invention involves
the use of molecules which modify the T cell response
without modifying the B cell response. Such materials

15   are especially useful as vaccines, as discussed below.
The molecules of the invention are preferably, but
not exclusively, superantigen derivatives. These
derivatives may be modified or mutated, as discussed
above. These, or any other molecules used herein, are

20   administered in an amount sufficient to modify the T
cell response in the manner described. The amount of
material used will vary, depending on the actual
material, the response desired, and the subject matter
of the treatment.

25   The molecules may also serve as vaccines. These
vaccines confer protective immunity on the subject in
that they generate a B cell response without the full T
cell response normally associated with the normal form
of the molecule. Example 7 shows one manifestation of

30   this effect for SEB. Again, depending upon the
parameters within the control of the knowledge of the
artisan, including the condition being treated, the Vβ
molecule to be regulated, and so forth, the material
chosen for the vaccine is up to the artisan. The

35   vaccine may contain other materials which are normally
found in vaccine compositions, including adjuvants,
carriers, etc.

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The mode of administration of the materials
described herein may vary as well, including
intravenous, intraperitoneal, and intramuscular
injections, as well as all of the other standard

5   methods for administering therapeutic agents to a
subject.

The invention also discloses how to make
particular mutants useful in the foregoing
methodologies, including isolated nucleic acid

10   sequences coding for mutants, cell lines transformed by
these and the vectors and plasmids used therefor, as
well as the isolated mutant molecules, including mutant
superantigens.

Other applications of the invention described

15   herein will be apparent to the skilled artisan and need
not be repeated here.

The terms and expressions which have been employed
are used as terms of description and not of limitation,
and there is no intention in the use of such terms and

20   expressions of excluding any equivalents of the
features shown and described or portions thereof, it
being recognized that various modifications are
possible within the scope of the invention.

Polymerase Chain Reaction (PCR) and standard

25   molecular biological methodologies, described in
Example 1, were used in the construction and expression
of recombinant SEB. SEB mutants were generated in one
of two ways, as described in Example 2. The first way
introduced random mutations along the entire length of

30   the SEB gene. A second method introduced random
mutations in approximately 60-75 base-defined regions
of the SEB gene. Initial identification of potential
mutant SEBs tested the lysate from transformants for
the presence of functional toxin by stimulation of

35   murine T cell hybridomas bearing different $V\beta$ elements
in a human DR-expressing cell line. Lysates negative
for T cell hybridoma stimulations were tested for the

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presence of SEB with the use of monoclonal antibodies
(mAbs) against SEB (Example 3). Transformants
producing non-functional SEB were sequenced and the
mutation identified. Transformants producing mutant
5   SEBs were grown, mutant SEBs purified as described in
Example 4. Analysis of the location and effect of the
mutation was performed. Since binding to MHC class II
molecules is a prerequisite for toxin recognition by T
cells, the ability of mutant SEBs to bind human MHC
10   antigen HLA-DR1 was tested as described in Example 5.
Mutant SEBs, such as region 3 mutants, were produced
which selectively stimulate some, but not all, of the
hybridomas bearing specific Vβ elements stimulated by
the non-mutated toxin. Thus, the mutated superantigens
15   of the present invention may be used to selectively
stimulate only some of the T cell populations
stimulated by the wild-type superantigen.

         Three regions were identified in the N-terminus
part of SEB that affect MHC and/or TCR binding (Example
20   4 and Figure 1). Mutations in region 1 (residues 9-23)
affected both MHC and TCR binding. The results
suggested that 23N was particularly important. When
the sequences of the S. aureus enterotoxins are aligned
for maximum homology (Marrack & Kapple (1990) supra),
25   this residue is conserved among all of the enterotoxins
and toxic shock toxin as well. The mutations in region
2 (residues 41-53) drastically reduced the ability of
the toxin to bind to MHC class II with a similar effect
on their ability to stimulate T cells. About half of
30   the mutations involved F44. Again, this residue is
conserved among all the enterotoxins, indicating that
this residue probably plays a critical role in the
binding of all of the toxins to MHC. Interestingly,
none of the mutations in either region 1 or 2
35   completely obliterated toxin binding to MHC, and in
both cases the T cell-stimulating ability of the
mutants could be recovered by adding a large excess of

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toxin. Mutations in region 3 (60N, 61Y) did not affect
binding of the toxins to MHC, but did affect their
interaction with two Vβs, 7 and 8.1. This Vβ-specific
effect suggests that these amino acids are important
for interaction with some, but perhaps not other, TCR
Vβs.

The toxicity of mutant SEBs in animals was tested
as described in Example 6. Mice were injected with
either balanced salt solution (BSS), recombinant SEB,
or region 1 SEB mutants at F44 (BR-358) or at N23 (BR-
257). Mice receiving 50 ug of either mutant SEB were
indistinguishable from those receiving BSS, while those
receiving recombinant SEB died within 5 days.

The ability of mutant SEB to provide immune
protection from SEB was tested in vivo (Example 7).
Mice receiving 100 ug of mutant SEB BR-257 three months
prior to challenge with SEB were fully protected from
the toxic effect of SEB, whereas those animals not
injected with BR-257 died 4-5 days after challenge with
SEB. Similar results were obtained with primates.
Mutant SEB BR-358 and BR-257 were either ineffective or
much less effective in eliciting an emetic response in
monkeys (Example 7).

Example 8 describes the application of the above
procedure to the SEA toxin and the production of a SEA
mutant which behaves the same as the corresponding SEB
mutant.

Example 1.   Construction and Expression of
               Recombinant SEB.

Polymerase Chain Reaction (PCR). PCRs (Saiki et
al. (1988) Science 239:487) were performed using
AmpliTaq recombinant Taq polymerase and the DNA Thermal
Cycler from Perkin Elmer Cetus (Norwalk, CT). 20-30
cycles were performed with 1-min denaturing and
annealing steps, and an extension step of 1 min for
synthesis < 500 bp and 2 min for those > 500 bp.

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Template concentrations were 1-10 nM and
oligonucleotides primer concentrations were 1 uM. The
concentration of the dNTPs was 200 uM, except when
attempting to introduce mutations, where the
5   concentration of one of the dNTPs was reduced to 20 mM.
SEB Construct. The gene for superantigen SEB was
overexpressed in E. coli as follows. A linearized
plasmid containing the genomic SEB gene (Ranelli et al.
(1985) Proc. Natl. Acad. Sci. USA 82:5850) was used as
10   a template in a PCR utilizing oligonucleotide primers
that flanked the portion of the gene encoding the
mature SEB without the signal peptide. The 5' primer
was (SEQ ID NO:1):

15   5'-TAGGGAATTCCATGGAGAGTCAACCAGA-3'

This primer contains an EcoRI site which places
the SEB gene in-frame with the LacZ gene when cloned
into plasmid pTZ18R (Pharmacia Fine Chemicals,
20   Piscataway, NJ). This oligonucleotide primer also
contains an NcoI site which adds an ATG between the
LacZ gene fragment and the beginning of the SEB gene so
that the SEB gene could be moved easily to other
plasmids carrying its own initiation ATG. The 3'
25   primer contained a HindIII site after the termination
codon of the SEB gene (SEQ ID NO:2):

                     5'-AGCTAAGCTTCACTTTTTCTTTGTCG-3'

30   The PCR fragment was digested with EcoRI and
HindIII and ligated into EcoRI/HindIII-digested pTZ18R.
E. coli XL1-Blue (Stratagen, La Jolla, CA) was
transformed with the plasmid, a single transformant
picked, and the insert (pSEB2) was sequenced to check
35   that it had no mutations.
Upon induction the pSEB2 construct led to
overproduction of mostly cytoplasmic SEB ($\approx$ 10 ug/ml of

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broth). However, rather than producing a LacZ/SEB
fusion protein, the bacteria produced a protein with
the same apparent molecular weight as secreted SEB from
S. aureus (Fig. 1a). Either the LacZ portion of the
fusion protein was cleaved in vivo from the majority of
SEB or the ATG introduced between LacZ and SEB was a
more efficient translation initiation site than that of
LacZ.

Example 2.   Generation of SEB Mutants.

    SEB mutants were generated in one of two ways.
One way introduced random mutations along the entire
length of the SEB gene. To do so, the SEB construct of
Example 1 was prepared but PCR was performed with
concentrations of either dATP or dTTP reduced 10 fold
in order to increase Taq polymerase error rate (Innis
et al. (1988) Proc. Natl. Acad. Sci. USA 85:9436).
This reduces the product amount 5-10 fold. Products of
the two reactions were combined, cloned into pTZ18R as
described in Example 1, and individual transformants
were screened for mutant SEB as described in Example 3
for BR mutants. Of approximately 400 toxin-producing
transformants screened, 10 were identified as
functional mutants by their reduced ability to
stimulate T cells. Low concentrations of dCTP and dGTP
were tried as well, but less reduction in product
results and no mutants were detected in screening
approximately 200 transformants.

    A second PCR method was used for introducing
random mutations in approximately 60-75 base-defined
regions of the SEB gene. The following oligonucleotide
primers (A (SEQ ID NO:3), B (SEQ ID NO:4), and C (SEQ
ID NO:5)) positioned as shown in Figure 2, were
synthesized with each position containing 1% each of
the three incorrect bases:

A-:   5'ATTCCCTAACTTAGTGTCCTTAATAGAATATATTAAGTCAAAGTATAG
      AAATTGATCTATAGA3'

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B-:   5'AGCTAGATCTTTGTTTTTAAATTCGACTCGAACATTATCATAATTCCC
GAGCTTA3'

C+:   5'CCGGATCCTAAACCAGATGAGCTCCACAAATCTTCCAATTCACAGGCC
TGATGGAAAATATGAAAGTTTGTAT3'

These mutant oligonucleotides served as primers in
a PCR reaction with either a vector (A and B) or
internal SEB (c) oligonucleotide as the other primer,
and the SEB gene as the template. Each molecule of
synthesized SEB fragment was predicted to have 2-3
random base mutations in the region corresponding to
mutant primer. Mutant fragments were incorporated into
the SEB gene, either alone or with another fragment
containing the 3'-portion of the gene as mixed template
in a PCR reaction to resynthesized a full length SEB2
gene (Ho et al. (1989) Gene 77:51; Pullen et al. (1990)
Cell 61:1365). Alternatively, this was accomplished by
digestion with appropriate restriction enzymes and
ligation into pSEB2 from which the corresponding region
had been removed.

DNA Sequencing. Plasmid inserts were sequenced
directly by the dideoxynucleotide method of Sanger et
al. (1977) Proc. Natl. Acad. Sci. USA 74:5463, using
Sequenase (U.S. Biochemical Corp., Cleveland, OH) and a
modification for double-stranded supercoiled plasmid
templates (Weickert and Chambliss (1989) in Editorial
Comments, U. S. Biochemical Corp., Cleveland, OH, pg.
5-6.

Example 3.   Screening of Transformants for SEB
Mutants.
Anti-SEB Monoclonal Antibodies (mAbs). 10 mAbs
specific for at least five epitopes of SEB were
produced by standard methods from B10.Q(βBR) immunized
multiple times with SEB. One of these antibodies,
B344.1, was used both for quantitation and
immunoaffinity purification of SEB and SEB mutants.
B344.1 is an IgG1 that was chosen because initial

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characterization showed that it had a high affinity for
SEB, bound equally well to all of the SEB functional
mutants, could detect and immunoprecipitate SEB bound
to MHC class II molecules, and did not block T cell
5   recognition of SEB bound to DR (data not shown).

ELISA for SEB. The amount of SEB in preparations
wad determined by ELISA. Microtiter wells were coated
overnight with a solution of 6 ug/ml natural SEB (Sigma
Chemical Co., St. Louis, MO). The wells were then
10   incubated with 25% FCS and washed throughly. Various
concentrations of known and unknown SEB preparations
were added to the wells as inhibitor followed by a
constant amount of anti-SEB antibody (polyclonal rabbit
anti-SEB(Toxin Technology, Madison, WI) in BR
15   experiments and monoclonal anti-SEB, B344, in BA, BB,
and BC experiments). After 1 hour, the wells were
washed thoroughly, and the bound antibody was detected
by standard techniques using alkaline phosphatase
coupling either to goat anti-rabbit IgG (Sigma Chemical
20   Co.) or to p-nitrophenyl phosphate as substrate. The
OD of the reaction at 405 nm was related to the dose of
inhibitor and the concentration of the SEB in the
unknown estimated by computer analysis of the data.

Initial Screening of Potentially Mutant SEB. For
25   primary screening, totaly lysates were prepared as
described from individual transformants containing a
potentially mutant SEB gene. Aliquots of each lysate
were tested for the presence of functional toxin by
stimulation of murine T cell hybridomas bearing α/β
30   receptors with either Vβ7 or Vβ8.3, using human DR-
expressing cell lines as presenting cells. Lysates
deficient in stimulating either of these hybridomas
were assayed for the presence of SEB protein to rule
out mutations affecting the level or the full length of
35   the SEB produced. Plasmids from those producing
proteins were sequenced to locate the mutation the
sequences of the mutants are shown in Figure 2.

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The Taq polymerase error-induced random mutants
(BR) were clustered in three regions (1, 2, 4), all in
the NH$_2$-terminal 93 amino acids of the molecule (except
an additional conservative mutation in one case, BR-
374, in the COOH-terminal half of the molecule). As
predicted by their method of generation, all but one of
these mutations involved a nucleotide substitution of A
to G or T to C, and only one silent mutation was found
elsewhere in their sequences (data not shown).
Additional mutants were generated in region 1 or 2 with
mutant oligonucleotide C or A (BC, BA mutants). Region
3 was originally discovered as a single mutant (BA-62)
involving the last amino acid covered by
oligonucleotide A. The mutant had a different
phenotype than the other BA mutants. Additional
mutants were produced in this region with mutant
oligonucleotide B (BB mutants). Mutations in region 4
were eliminated from further analysis, because it was
felt that interfering with the conserved disulfide
forming cysteine at position 93 could have far reaching
unpredictable effects. In addition, several mutants
were not further characterized either because they
involved more than one region (Br-474, BA-72), produced
highly degraded toxin (BR-267), or were identical to an
already existing mutant (BA-50).

Example 4.   <u>Preparation of Recombinant SEB</u>.
     For initial screening, individual colonies of
transformants picked from agar plates were transferred
to wells of 96-well microtiter plates containing 100 ul
of 2XYT and carbenicillin. A replicate plate was
prepared except that the media contained 1 mM IPTG as
well. Both were incubated overnight at 37°C with
shaking. 50 ul of glycerol was added to each well of
the first plate, which was mixed and then stored at -
70°C. To prepare SEB-containing lysates, each well of
the second plate received 50 ul of HNM buffer (10 mM

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Hepes, pH 7.0, 30 mM NaCl, 5 mM MgCl$_2$) containing 3
mg/ml lysozyme and 300 ug/ml DNAse I. The plate was
incubated at 37°C for 15 min, frozen, thawed three
times, and centrifuged to pellet debris. The
5   supernatants were transferred to a new plate and tested
for the presence of SEB both by ELISA and T cell
hybridoma stimulation. This method produced
preparations containing 0.3 and 10 ub/ml of SEB.
     To produce purified mutant SEB, transformants were
10   recovered from the 96-well plate stored at -70°C.
Bacteria from overnight cultures (1 vol) containing
IPTG were collected by centrifugation, resuspended in a
1:10 vol of HNM buffer containing 1-2 mg/ml lysozyme
and 10 ug/ml DNAse I, and frozen and thawed three
15   times. The suspension was centrifuged at 15,000 g for
20 min to remove bacterial debris, and the supernatant
was harvested and filtered (0.2 u). The filtrate was
passed through a column containing a 1:50 volume of
Sepharose 4B beads coupled with 2-3 mg/ml of a mAb to
20   SEB (B344). The beads were washed thoroughly with PBS
and the toxin was eluted with 0.1 M glycine-HCl (pH
2.7) and neutralized with 1 M Na$_2$CO$_3$. The SEB was
concentrated to 1 mg/ml and its buffer changed to BSS
using Centricon10 microconcentrators (Amicon Corp.,
25   Denvers, MA). This method yielded 3-10 mg of toxin per
liter of bacterial culture. SEB and its mutants
produced in this manner were > 95% pure as judged by
SDS-PAGE. Region 1 SEB mutants are listed in Table II,
region 1 and 2 mutants are listed in Table III, and
30   region 3 mutants are listed in Table IV.
     The mutations described all involve a nucleotide
substitution of A to G, or T to C, which would be
predicted by the methodology used for their generation.
     When mutations were generated using mutant
35   oligonucleotides C or A, these mutations were
concentrated in amino acids 9-23 (Region 1), or 41-53
(Region 2) of the SEB sequence (Table III).

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When the oligonucleotide primer B was used, the
mutants listed in Table IV were generated.

Amino acid 93 is cysteine in normal SEB. To that
end, mutations in this region were not considered
5   further because of the potential interference with
disulfide binding. Thus, mutants BR-30 and BR-311 were
eliminated.

Those mutants containing changes in more than one
region (NOT more than one mutant), i.e., BR-474 and BA-
10   72, were also eliminated, as was BR-267, because the
toxin was highly degraded. BA-50 is a known mutant and
was not studied further.

Structural Studies of SEB.

15   The three-dimensional crystal structure of SEB,
perhaps the most widely studied member of the
staphylococcal enterotoxins, has been recently reported
(Swaminathan et al. (1992) Nature 359:801). A
schematic drawing of SEB is shown in Figure 1. The SEB
20   molecule contains two domains. The first is composed
of residues 1-120 and the second of residues 127-239.
As discussed above, three regions have been identified
(Kappler et al. (1992) J. Exp. Med. 175:387) in the N-
terminus part of the SEB that affect MHC class II
25   binding and/or T cell activation. In each of the
regions the specific amino acids that are responsible
were determined. Some of the identified residues
affect both MHC class II binding and T cell activation,
whereas other affect only the latter. As superantigen-
30   MHC class II binding is a prerequisite for T cell
activation, residues affecting MHC class II binding
will also influence T cell activation, thus no T cell
binding information can be inferred from them. But
they do provide information about MHC class II binding
35   sites on the superantigen. On the other hand, those
residues that influence T cell activation but not MHC

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class II binding are likely to be in the T cell binding
site on SEB.

   Region 1, defined as the stretch of amino acid
residues from 9-23, is bifunctional as it affects both

5   TCR and MHC class II binding. Mutations in this region
included residues in positions 10, 14, 17 and 23, as
either a single or a double mutation (Table II).
Asparagine at residue 23 (N23) is on the α-helix, α2,
with the side chain pointed towards the solvent. It is

10 the most important residue, being conserved among all
staphylococcal enterotoxins and critical for TCR
activation. Only five mutations at position 23
affected MHC class II binding, but all of them affected
TCR activity. Mutations at residue 14 and 17 affected

15 both MHC class II binding and TCR activation. Residue
S14 is on a very short α-helix (α1) and is exposed to
the solvent whereas F17 is located at the other end of
α1. The locations of S14, F17, and N23 (Figure 1) on
the surface of the toxin are favorable for making

20 critical contacts with MHC class II molecules and/or
Vβ. Residue F17 points inwards and lies in a loop
sandwiched between two other loops, of residues 174-179
and 203-209. This suggests that its replacement by
serine (F17S) may have introduced structural changes

25 which reduce MHC class II binding and cytokine
production. The association of S14 (on α1) with MHC
class II binding, and N23 (on α2) with TCR activity,
reveals the structural basis underlying the
bifunctional role of region 1. Although the region

30 consists of a small number of sequential amino acids,
there are distributed on separated but adjacent
elements of the secondary structure that are engaged in
different functions. The proximity of α1 and α2 is
consistent with the suggestion (Kappler et al. (1992)

35 supra) that the amino acids in region 1 are situated in
the trimolecular complex near the junction of Vβ and
MHC class II.

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Region 2 is defined as residues 40-53 and was
suggested to be important in all staphylococcal
enterotoxins in mediating binding to MHC class II.
About half of the mutations in this region involved the
conserved residue F44 (Table III). Other mutations
involved residues 41, 45, 48, 52, and 53. These
changes affected MHC class II binding and consequently
the TCR activation. Thus, this region is probably
specific for MHC class II binding. Residues 48-52 are
in β-strand β2. Residue F44 is on a turn connecting β1
and β2 with the side chains exposed to solvent. It is
situated favorably for engaging in critical hydrophobic
binding contacts with MHC class II.

Region 3 is made up of two residues, 60 and 61,
and mutation of either one affects the TCR activation
but not MHC class II binding. Residues 60 and 61 are
in the loop connecting β2 and β3 and are exposed to
solvent (Table IV).

Example 5.   Binding of Mutant SEB to HLA-DR.

Since binding to MHC class II is a prerequisite
for toxin recognition by T cells, the mutations could
have affected either the ability of the toxin to bind
to DR molecules or the recognition of this complex by
the TCR-α/β. To help distinguish these two
possibilities, the HLA-DR1 homozygous lymphoblastoid
line LG2 was used (Gatti and Leibold (1979) Tissue
Antigens 13:35). ^{125}I-labelled LG2 cells were incubated
with or without 50 ug/ml recombinant SEB for 2 hours at
37°C. A cell free lysate was prepared in 1% digitonin
and incubated for 4 hours at room temperature with
Sepharose beads coupled with 3 mg/ml B344 anti-SEB mAb.
The beads were washed thoroughly, and the labeled bound
material was analyzed by SDS-PAGE under reducing
conditions (Laemmlli (1970) Nature 227:680) and
autoradiography. As a control, beads bearing the anti-
DR mAb, L243 (Lampson and Levy (1980) J. Immunol.

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125:293) were used (1/20 the volume of lysate used with
the anti-SEB beads).

SEB binds to DR molecules on LG2 (Figure 2b).
Immunoaffinity-purified toxins were prepared and
assessed for their ability to bind to LG2 using flow
cytometry with the same anti-SEB mAb used to purify the
SEB and its mutants. 3×10^4 LG2 cells were incubated
in 100 ul of tissue culture medium overnight at 37°C
with vrious concentrations of SEB or its mutants. The
cells were washed thoroughly and incubated for 30 min
at 4°C with approximately 1 ug/ml of the anti-SEB mAb,
B344.1. The cells were washed again and incubated for
15 min at 4°C with fluoresceinated goat anti-mouse IgG1
(Fisher Scientific Co.). The cells were washed again
and analyzed for surface fluorescence of the cells
corrected for the fluorescence seen with the secondary
reagent alone v.s the amount of toxin added. The
results, shown in Figure 4, are presented for mutations
in each of regions 1, 2, and 3.

The binding by four of the region 1 mutants to LG2
was indistinguishable from that of unmutated SEB. The
other three mutants were reduced in their binding
capacity by approximately 100-fold. These results
suggest that residues between 14 and 23 within region 1
are important in MHC binding. Five of the seven
mutations involved residue 23N. In only one case (BR-
291, 23N→S) did this mutation reduce MHC binding.
These results suggest residue 23N may be important in
both MHC binding and Vβ interaction. Region 2 mutants
all bound poorly to LG2, approximately 1,000 times
poorer than SEB, indicating that region 2 defines a
stretch of amino acids, especially 44F, important in
binding of the toxin to class II MHC. Region 3 mutants
were essentially unaffected in binding to LG2, strongly
suggesting that this two-amino acid region (60N, 61Y)
is important in Vβ interaction.

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Example 5.   <u>Effect of Mutations on T Cells Bearing</u>
              <u>Different Vβ Elements</u>.

The SEB mutants were originally identified because
they stimulated either a Vβ7⁺ or a Vβ8.3⁺ T cell
5   hybridoma poorly. To assess the effect of the SEB '
mutations on T cell recognition in more detail, the
·purified mutant toxins were retested at various doses
on additional T cell hybridomas bearing each of the
four murine Vβ elements known to recognize SEB (Vβ7,
10   Vβ8.1-3, (White et al (1989) <u>supra</u>; Callahan et al.
(1989) <u>supra</u>; Herman et al. (1991) <u>supra</u>). Varying
concentrations of toxins were incubated at 37·C
overnight with 3 x 10⁴ DR⁺ cells in 200 ul of tissue
culture medium. 5 x 10⁴ T cell hybridomas of requisite
15   Vβ specificity were added in 50 ul, and the mixture
incubated overnight. Response of T cell hybridomas was
measured as IL-2 secreted, following Kappler <u>et al.</u>
(1981) J. Exp. Med. <u>153</u>:1198 and Mosmann (1983) J.
Immunol. Meth. <u>65</u>:55. The results are shown in Figures
20   5-7.

Among the region I mutants (Figure 5), the five
involving 23N (BR-257, BR-291, BC-6, BC-66, BC-88)
stimulated all of the hybridomas poorly, despite the
fact that four of these bound to DR as well as
25   unmutated SEB did. These results indicate that residue
23N is an important amino acid for Vβ interaction, but
because the fifth mutant involving this amino acid, BR-
291, bound poorly to MHC, this amino acid may influence
MHC binding as well. The other two regions 1 mutants
30   also stimulated poorly. In the case of BR-75, this may
have been due primarily to its poor binding to DR, but
the effect of the BR-210 mutation was several orders of
magnitude greater on T cell stimulation than on binding
to DR. Taken together, these results are evidence that
35   during T cell recognition of SEB bound to DR, the amino
acids in region 1 are situated in the trimolecular

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complex at the junction between Vβ and MHC, so that
individual residues may interact with either component.

The mutations in the other regions produced less
complicated phenotypes. All of the region 2 mutants
were defective in stimulation of all of the T cell
hybridomas, regardless of the Vβ element in their
receptors (Figure 6). There were small differences,
but in general the effect of mutations on stimulation
was about the same as that seen on DR binding. These
results were consitent with the conclusion that
mutations in region 2 primarily affect DR binding.

The two-amino acid region 3 mutants were the most
discriminating (Figure 7). Despite the fact that
random mutants in a 20-amino acid strech flanking this
region were generated, all mutations affecting function
were found in these two amino acids. These mutants
failed to stimulate the hybridomas bearing Vβ7 and
Vβ8.1, but not Vβ8.2 or Vβ8.3. To insure that this
property was not peculiar to these particular
hybridomas, the toxins were tested with four other T
cell hybridomas: one Vβ7*. two Vβ8.1*, and one Vβ8.3*.
The results were indistinguishable from those in Figure
7 (data not shown).

Example 6. Requirement for T Cell Interaction for
In Vivo Effects of SEB.

The question of how important the superantigen
properties of the bacterial toxins are to their in vivo
toxic effects is unresolved. Previous experiments by
the inventors suggested that the toxicity of SEB in
mice was related to its ability to stimulate T cells in
a Vβ-specific manner, since the toxic effect of SEB was
directly related to the frequency of T cells bearing
the relevant Vβ elements (Marrack et al. (1990) J. Exp.
Med. 171:455). However, the ability of some of S.
aureus toxins to bind to class II on monocytes and
stimulate the production of cytokines such as TNF and

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IL-1 (Parsonnet (1989) Rev. Infect. Dis. 1:263) opens
the possibility that direct monocyte stimulation may be
sufficient to account for much of the toxin pathology
in some situations.

To test this idea, mice were injected with various
concentrations of region I mutant BR-257, which binds
very well to class II MHC but does not stimulate T
cells except at extremely high levels. Unmutated SEB
and mutant BR-358, which like all of the region 2
mutants binds very poorly to class II MHC, were used as
controls. To minimize the effects of LPS, which might
contaminate the preparations, C3H/HeJ mice were used, a
strain defective in LPS responsiveness. Since rapid
weight loss is one of the most obvious immediate toxic
effects of SEB in mice (Marrack et al. (1990) supra),
the mice were weighed daily after the injection on day
0.

Groups of three mice were weighed and then given
balanced salt solution (BSS) containing either nothing,
50 ug, or 100 ug of recombinant SEB, mutant SEB BR-257,
or mutant SEB BR-358. The mice were weighed daily at
the same time of day until they died. The results are
shown in Figure 8. Results are presented as the
average percent change from the starting weight for the
surviving mice.

Mice given either 50 or 100 ug of recombinant SEB
lost weight rapidly over 3-4 days, and all of the mice
were dead by day 5. Mice given mutant BR-358 showed no
effects and were indistinguishable from those given BSS
alone. Mice given 50 ug of BR-257 were unaffected as
well; however, those given 100 ug of BR-257 showed a
slight weight loss followed by recovery.

These results confirm that in mice the majority of
the toxicity of SEB is dependent on its ability to
stimulate T cells, suggesting that T cell-derived
lymphokines themselves or those produced by other cells
activated by T cells are very important in the mode of

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action of this toxin. However, the small effect of BR-257 at the higher dose raises the possibility of a contribution from class II-bearing cells directly stimulated by bound SEB without T cell involvement.

Example 7.   Protective Effect of SEB Mutants.

The protective effect of SEB mutants was tested. In these experiments, mice received doses of saline solution or 100 ug BR-257 three months prior to a challenge with wild-type SEB. On the day of the challenge (day "0"), the mice received 50 ug of SEB intraperitoneal. Weight change and survival were measured. Results are shown in Figure 9.

All mice which had received the control died 4-5 days after challenge with SEB, whereas there was a protective effect shown in the mice which had been immunized with the SEB mutant.

Example 8.   Production of SEA Mutants and Their
                  Protective Effects in Animals.

Staphylococcal enterotoxin A (SEA) mutants were produced according to the procedures described above. Superimposing the amino acid sequence of SEA on that of SEB, it has been bound that a mutation at position 45 inhibits SEA's ability to bind to MHC, in a similar manner to that observed with the position 45 SEB mutant.

Similar studies were conducted with primates. Monkeys received either wild-type SEB, or either of the reion 1 SEB mutants BR-257 (mutated at F44) or BR-358 (mutated at N23), and the induction of an emetic response assessed. Both mutant SEB molecules were either ineffective or much less effective in inducing an emetic response in primates, than wild-type SEB.

These results confirm that the method of producing mutant superantigen described in this disclosure is applicable generally to all superantigens, and provides

a method of protecting patients from the pathological
effect of superantigens.

TABLE I. KNOWN SUPERANTIGEN SEQUENCES AND STRUCTURES

Staphylococcus	Staphylococcal enterotoxin A	Huang et al (1987) J. Biol. Chem. 262:7006 Betley et al. (1988) J. Bacteriol. 170:34
	Staphylococcal enterotoxin B	Jones & Khan (1986) J. Bacteriol. 166:29 Huang & Bergdoll (1970) J. Biol. Chem. 245:3518 Ranelli et al. (1985) Proc. Natl. Acad. Sci. 82:5850
	Staphylococcal enterotoxin C1 and C3	Schmidt & Spero (1983) J. Biol. Chem. 258:6300 Bohach & Schlievert (1987) Mol. Gen. Genet. 209:15 Couch & Betley (1989) J. Bacteriol. 171:4507
	Staphylococcal enterotoxin D	Bayles & Iandolo (1989) J. Bacteriol. 171:4799
	Staphylococcal enterotoxin E	Couch et al. (1989) J. Bacteriol. 170:2954
Toxic Shock Toxin		Schlievert et al. (1981) J. Infect. Dis. 143:509 Blomster-Hautamaa et al. (1986) J. Biol. Chem. 261:15783 Bergdoll et al. (1981) Lancet 1:1017
Exfoliating Toxins		Lee et al. (1987) J. Bacteriol. 169:3904
Streptococcus	Streptococcal pyrogenic toxin C	Goshorn & Schlievert (1988) Infect. Immun. 56:2518 Tomai et al. (1990) J. Exp. Med. 172:359
Mouse Mammary Tumor Virus		Fasel et al. (1982) EMBO J. 1:3 Donehower et al. (1981) J. Virol. 37:226 Donehower et al. (1983) J. Virol. 45:941 Raceviskis & Prakash (1984) J. Virol. 51:604 Chol et al. (1991) Nature 350:203 Acha-Orbea et al. (1991) Nature 350:207 Pullen et al. (1992) J. Exp. Med. 175:41 Moore et al. (1987) J. of Virology 61:480

Fig 3 should this to be 9

TABLE II. REGION 1 SEB MUTANTS.

Mutant Name	Position	Change(s)
BR-75	F17	Phe-Ser
BR-210	S14	Ser-Leu
BR-257	10, N23	Asp-Asn; Asn-Asp
BR-291	N23	Asn-Ser
BR-358	F44	Phe-Ser
BR-374	D48, 160	Asp-Gly; Leu-Val
BR-30	Y91	Tyr-Cys
BR-311	C93	Cys-Arg
BR-474	46, C93	Tyr-Ser; Cys-Arg
BR-267	F44, 54, 55	Phe-Ser; Lys-Arg; Asp-Val

TABLE III. REGION 1 AND 2 SEB MUTANTS GENERATED WITH MUTANT OLIGONUCLEOTIDES A OR C.

Mutant Name	Position	Change(s)
BC-6	N23	Asn-Ile
BC-66	N23	Asn-Tyr
BC-88	N23	Asn-Lys
BA-3	F44	Phe-Cys
BA-15	L45	Leu-Val
BA-24	41, 53	Ile-Arg; Gln-Val
BA-31	46, 52	Tyr-Leu; Ser-Phe
BA-50	F44	Phe-Ser
BA-53	F44, 43	Phe-Leu; Ile-Arg
BA-62	Y61, 189	Gln-Ser; Ile-Arg
BA-72	L45, N60	Leu-Tyr; Asn-Lys

TABLE IV. REGION 3 SEB MUTANTS

Mutant Name	Position	Change(s)
BB-14	36, Y61	Gln-Leu; Tyr-Cys
BB-21	N60	Gln-Asn
BB-47	Y61	Tyr-Gln

CLAIMS

1.   A method for preventing the toxic effects of a superantigen by treatment with a molecule, wherein said molecule elicits antibody production without inducing T cell activation.

2.   The method of claim 1 wherein said molecule is a mutated superantigen.

3.   The method of claim 1 wherein said molecule is a modified superantigen.

4.   A molecule comprising a mutated superantigen.

5.   A molecule comprising a modified superantigen.

6.   A method of modifying T cell response elicited by an antigen comprising administering a molecule which interacts with specific Vβ elements of T cell receptors (TCR).

7.   The method of claim 6 wherein said molecule is a mutated superantigen.

8.   The method of claim 6 wherein said molecule is a modified superantigen.

9.   A method for treating the toxic effects of superantigen by treatment with a molecule, wherein said molecule elicits antibody production without inducing T cell activation.

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FIG. 1.

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FIG. 2B

FIG.2A.

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FIGURE 3

SEQ   ESQPDPXPDELIIKSSKFTGLNDMNMKVLYDXNIIVSAINVKSIDQFLYFDLIYSIKDTKLGNYDNYRVEFKNKDLADKYKDKYVDVFGANYYYQCYFS//QEED//I...I

IIR-75   N   S
IIR-210   L
IIR-257   D
IIR-291   S
DC-6   I
IIC-66   Y
IIC-88   K

IIR-358   S
IIR-374   G   V
IIA-3   C
IIA-15   R V   V
IIA-24   L   F
IIA-31   S   N
IIA-50   L   S
IIA-53   L   S   C

IIA-62   D
IIB-14   N
IIB-21
IIB-47

IIR-30   S   C
IIR-311   Y   R

IIR-474   S   R V   K   R
IIA-72   R
IIR-267
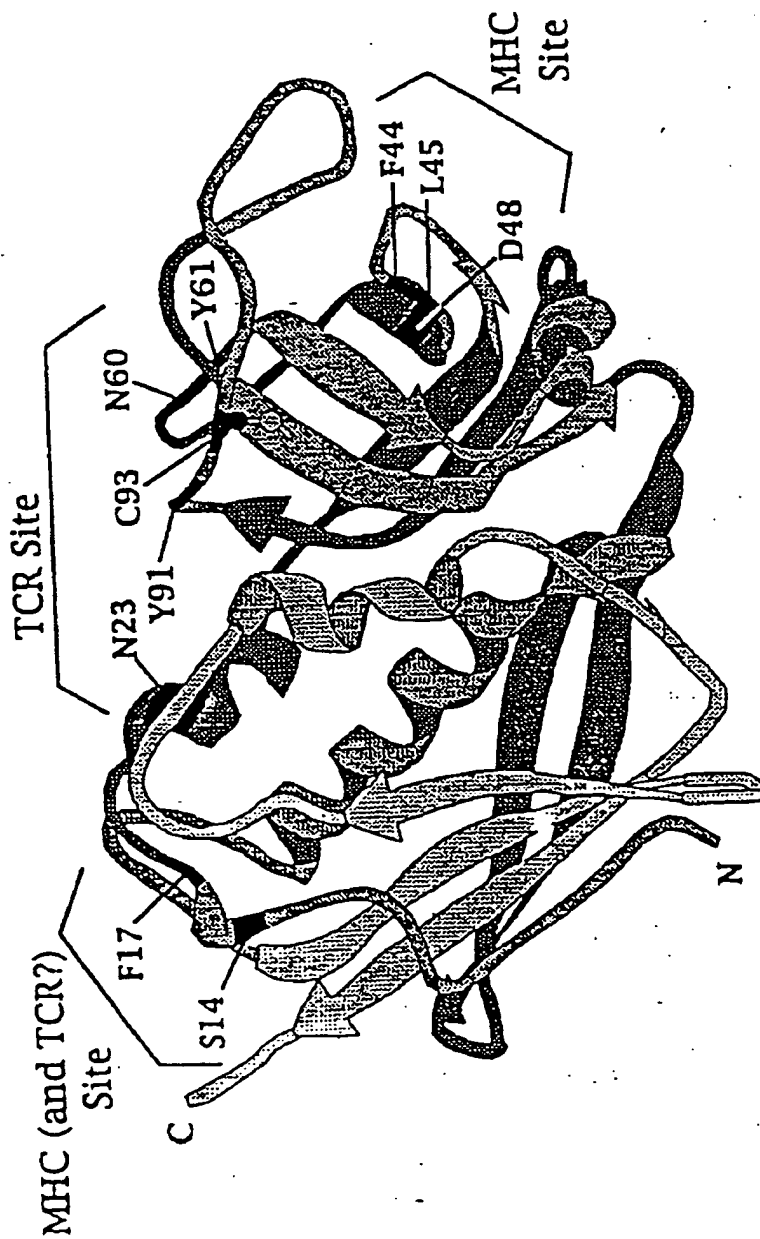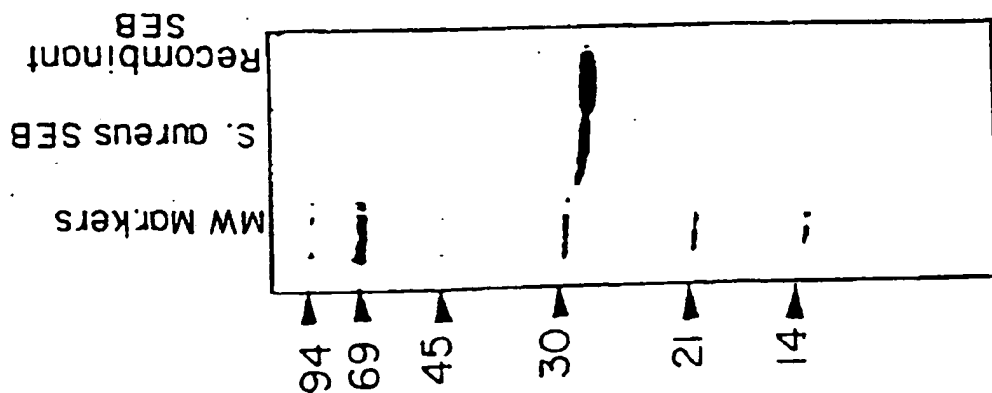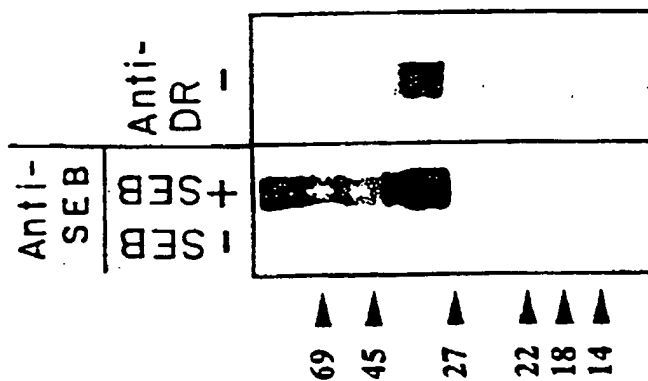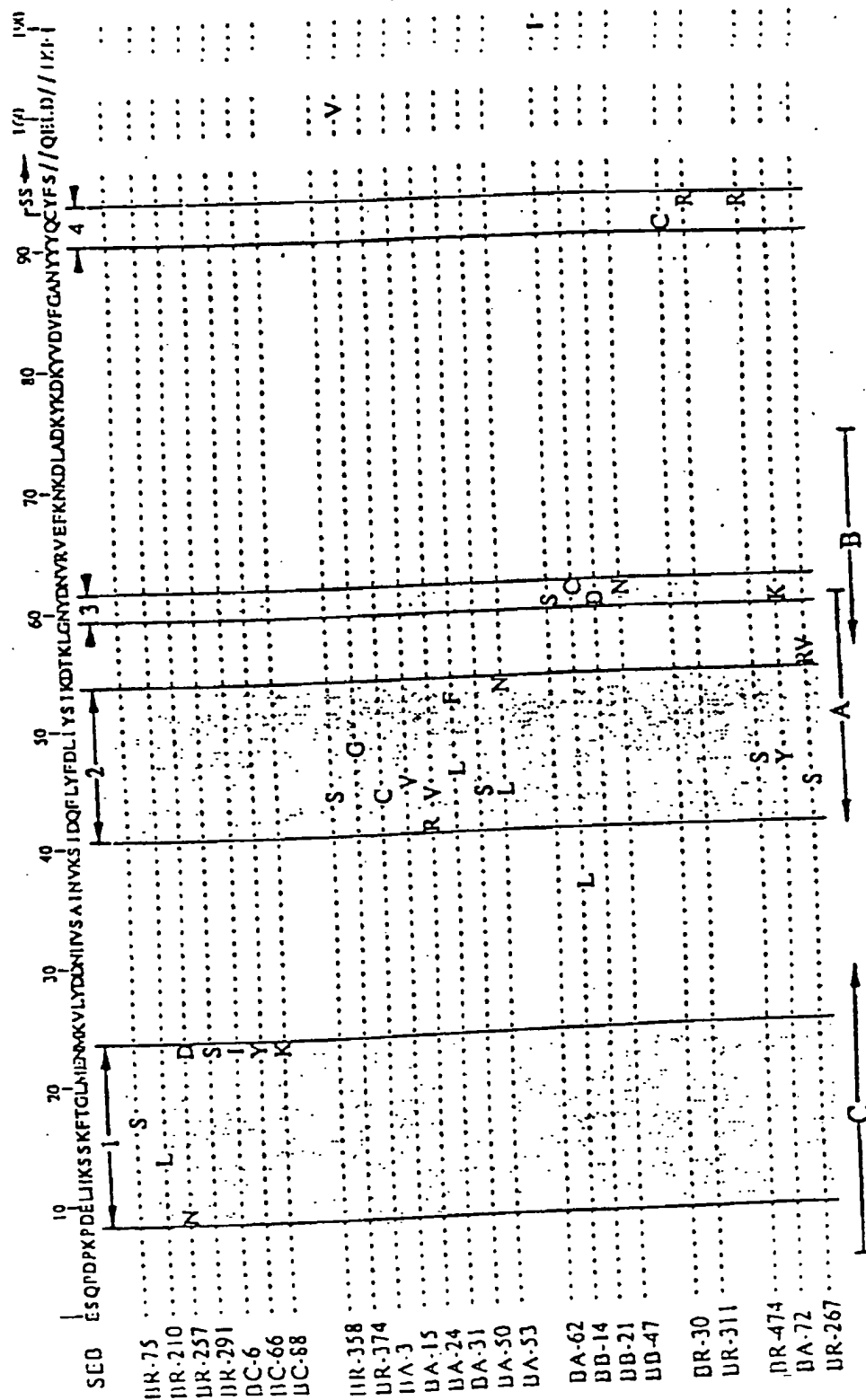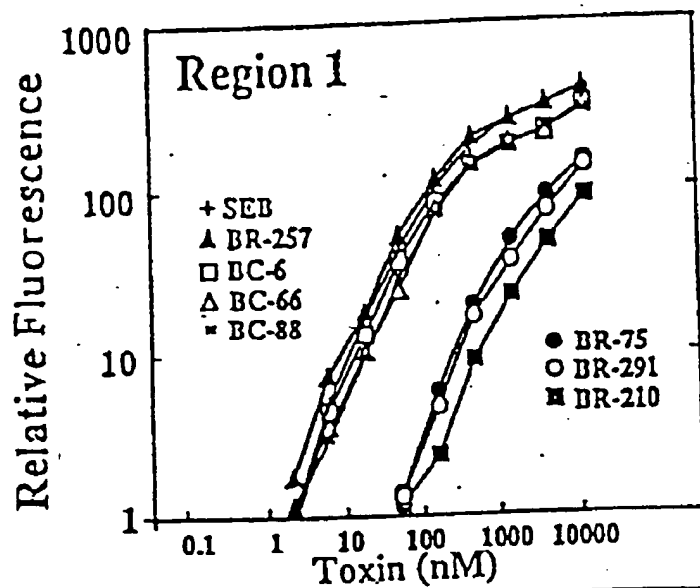
SUBSTITUTE SHEET

FIG.4A
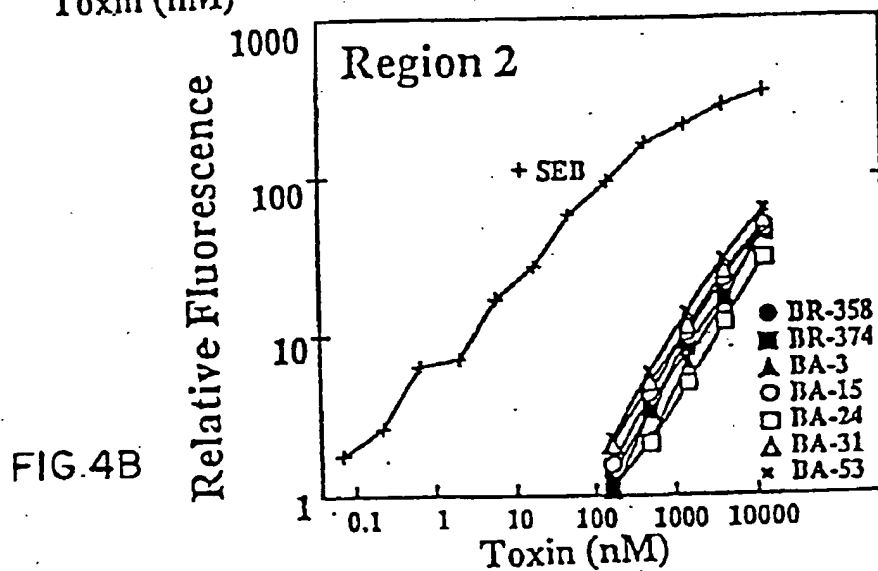
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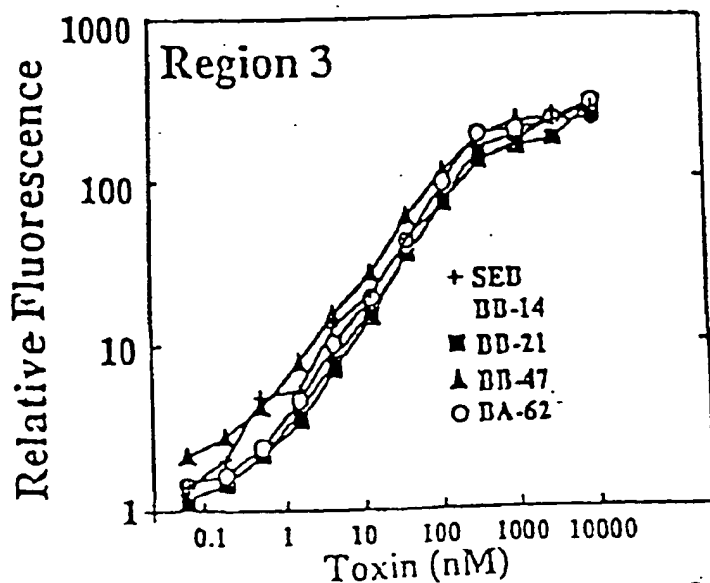
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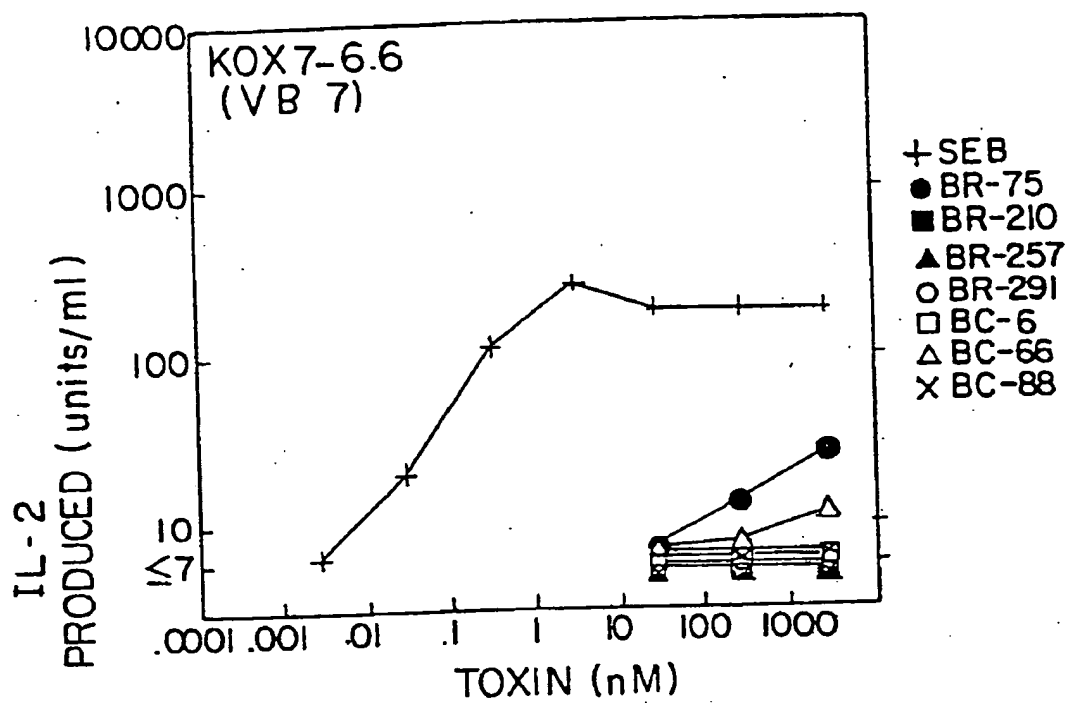
FIG. 5A
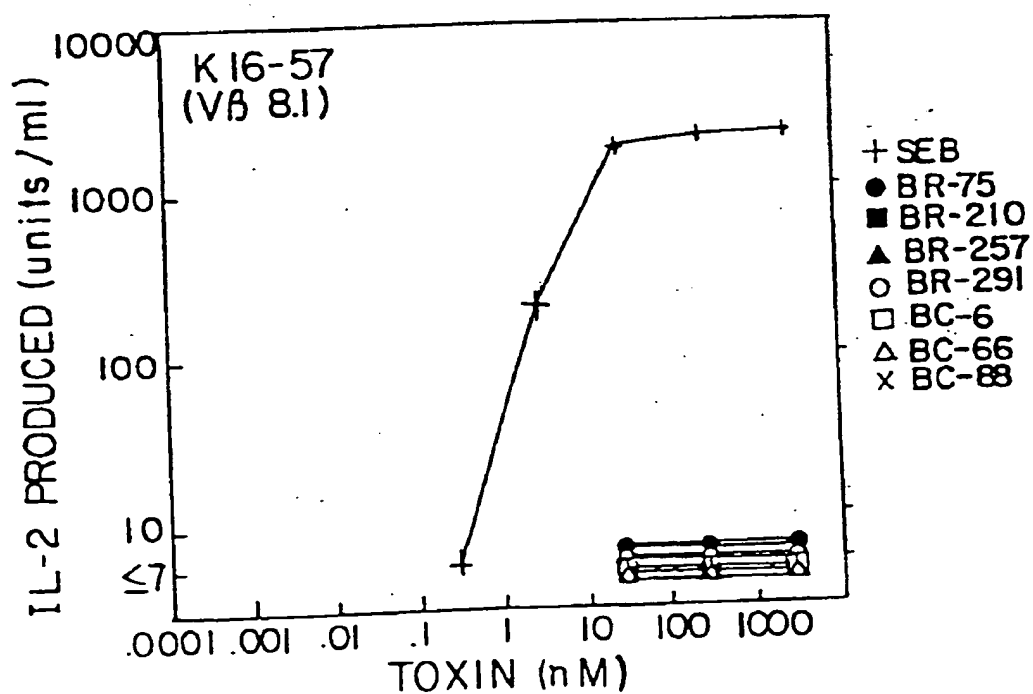
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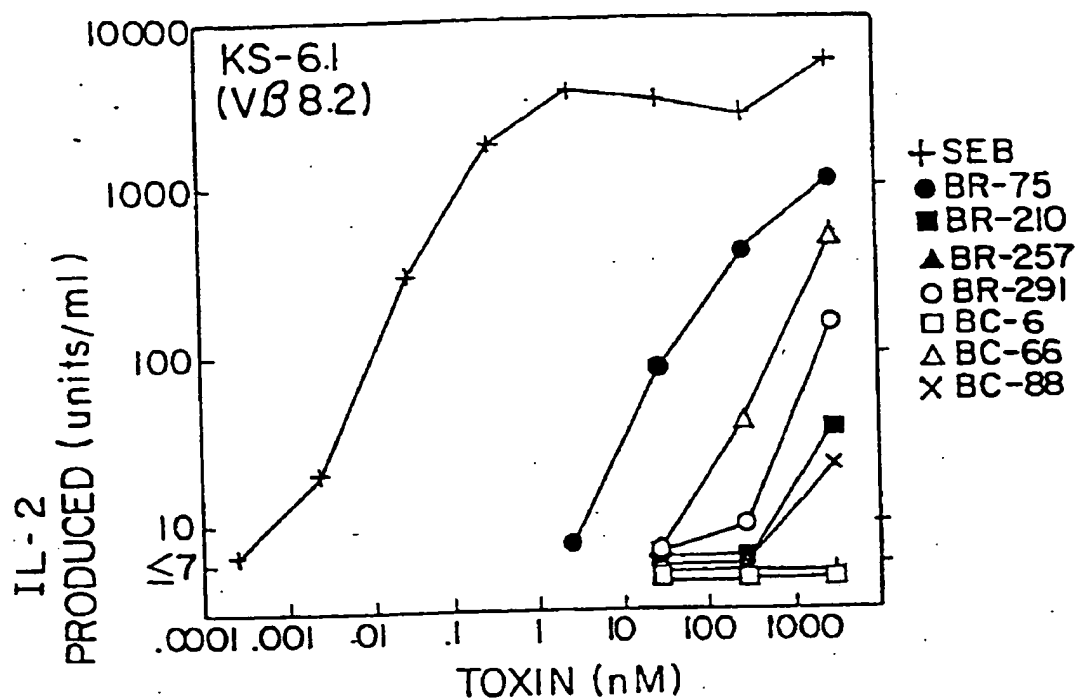
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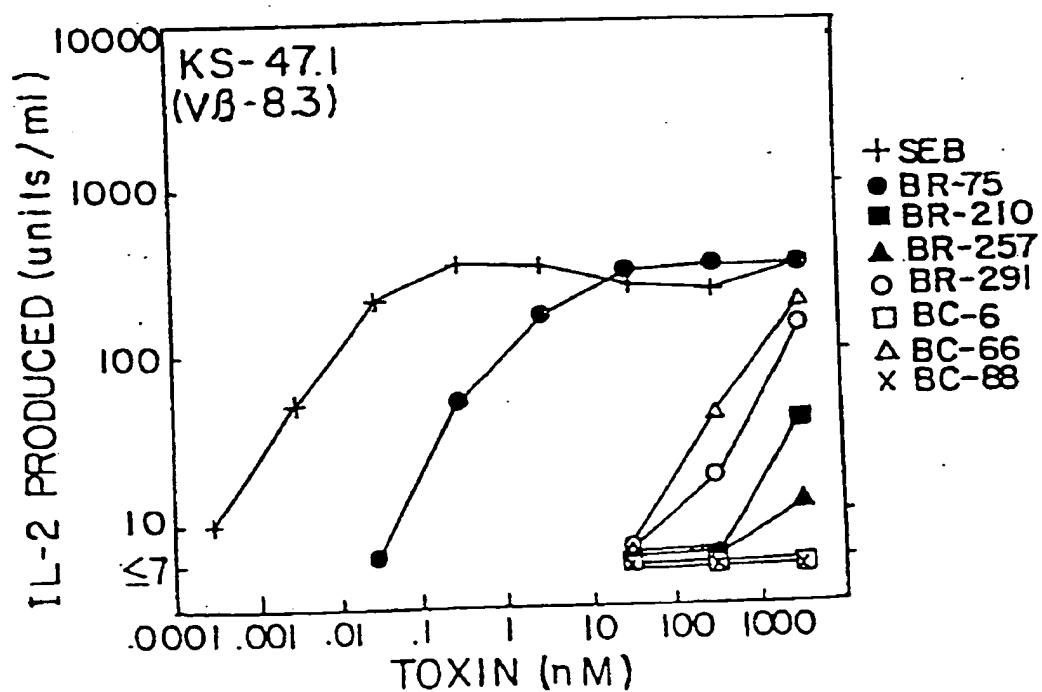
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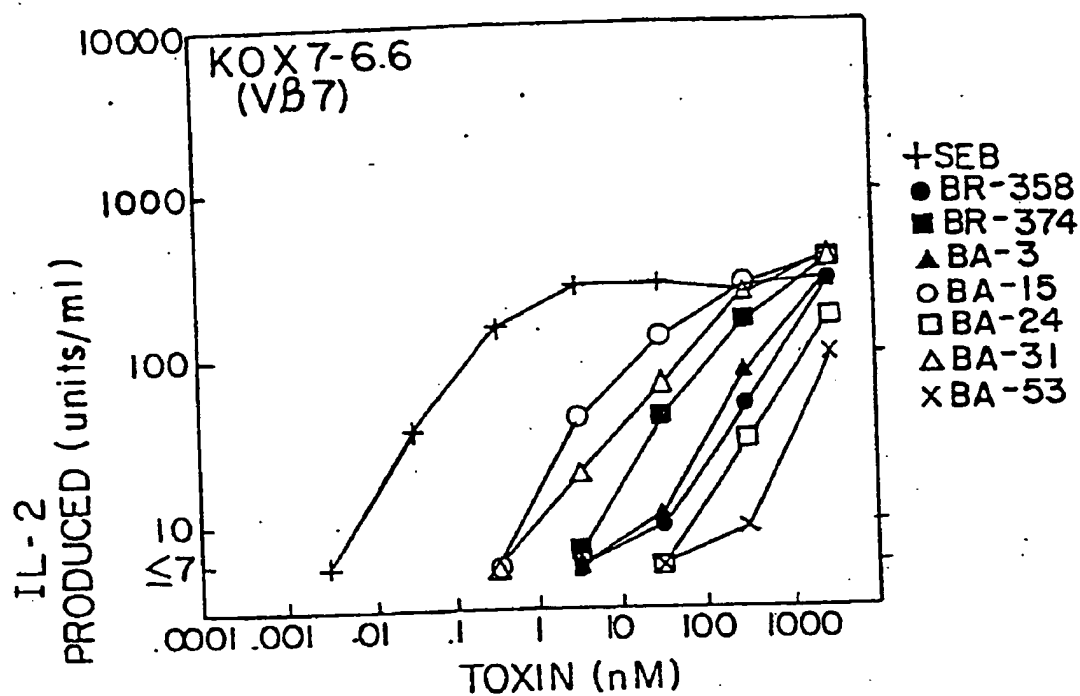
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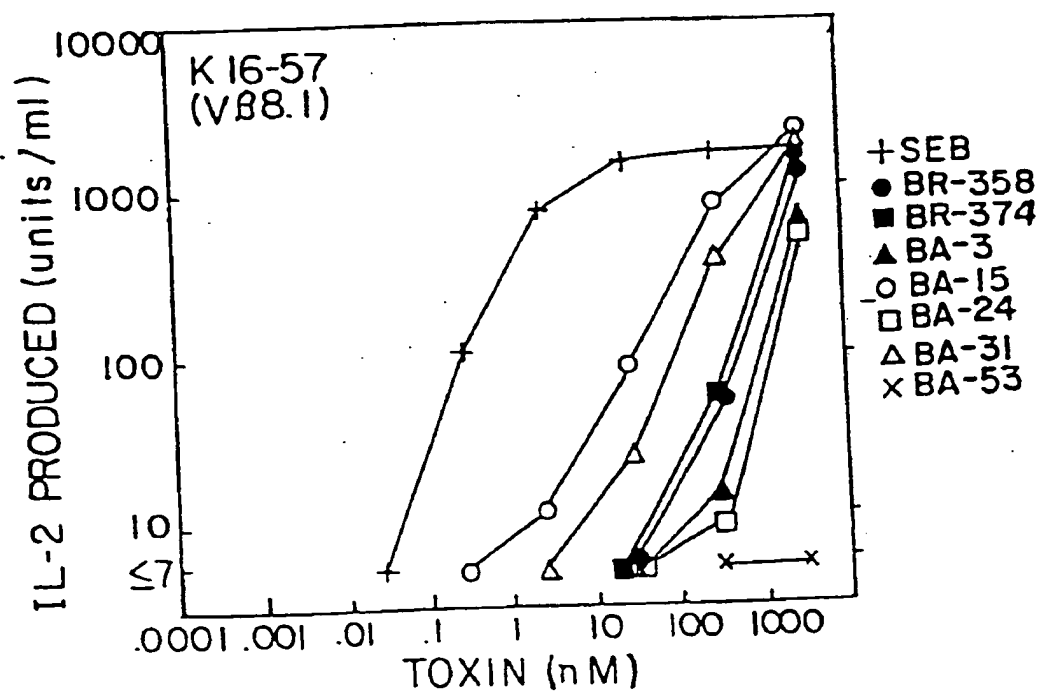
FIG. 6B
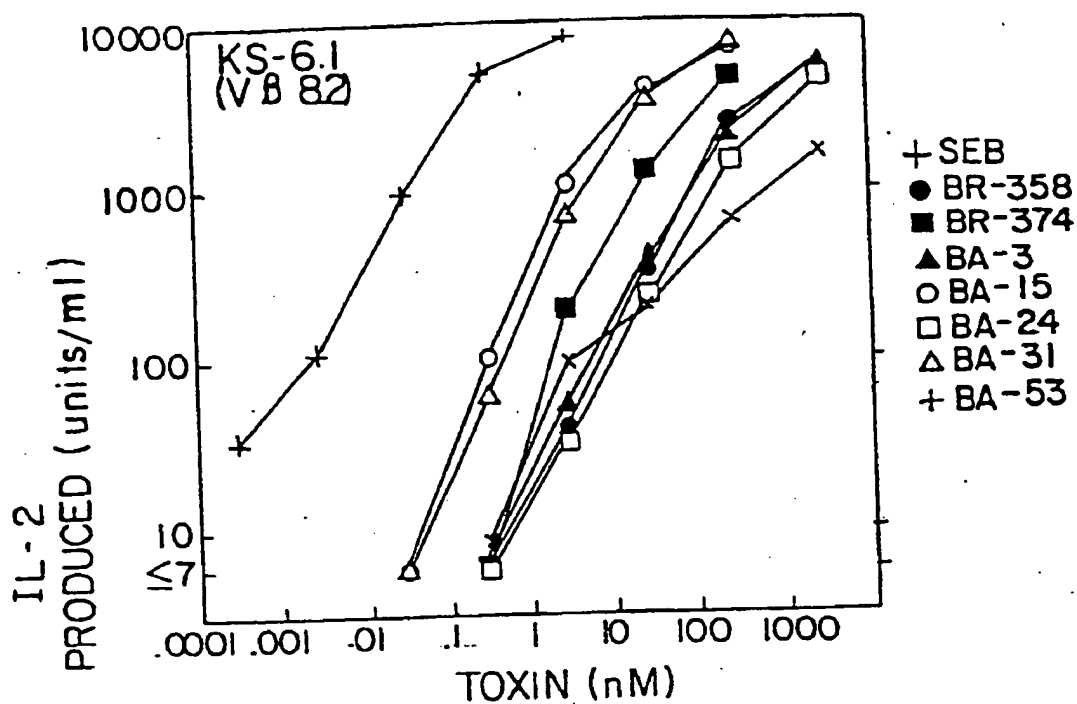
FIG. 6 C

FIG. 6 D

FIG. 7A

FIG. 7B

FIG.7C

FIG.7D

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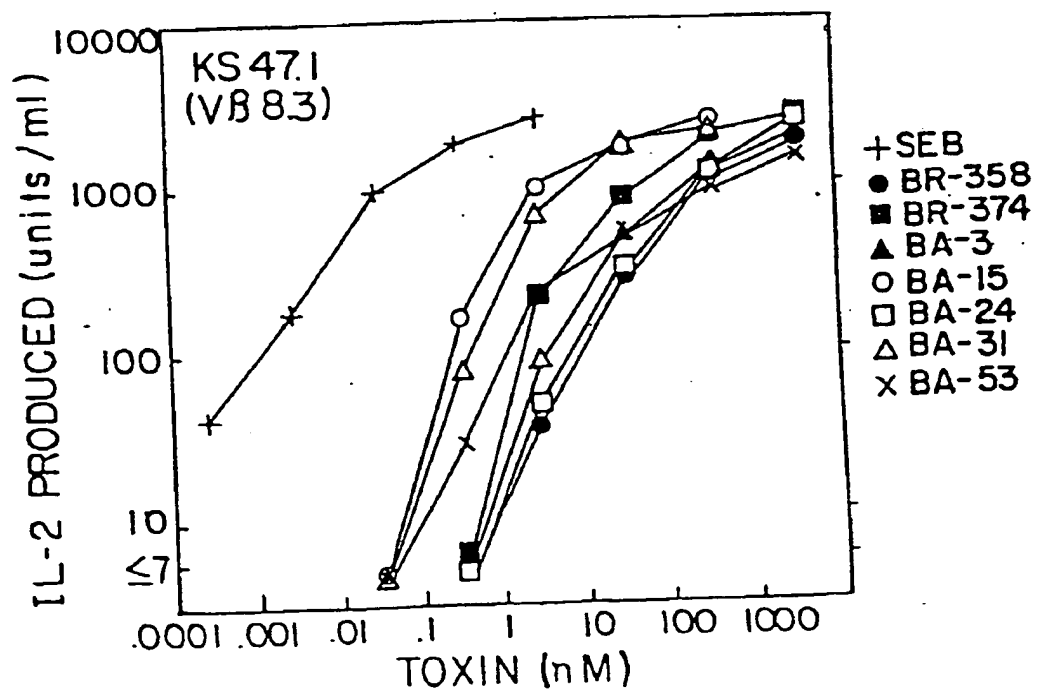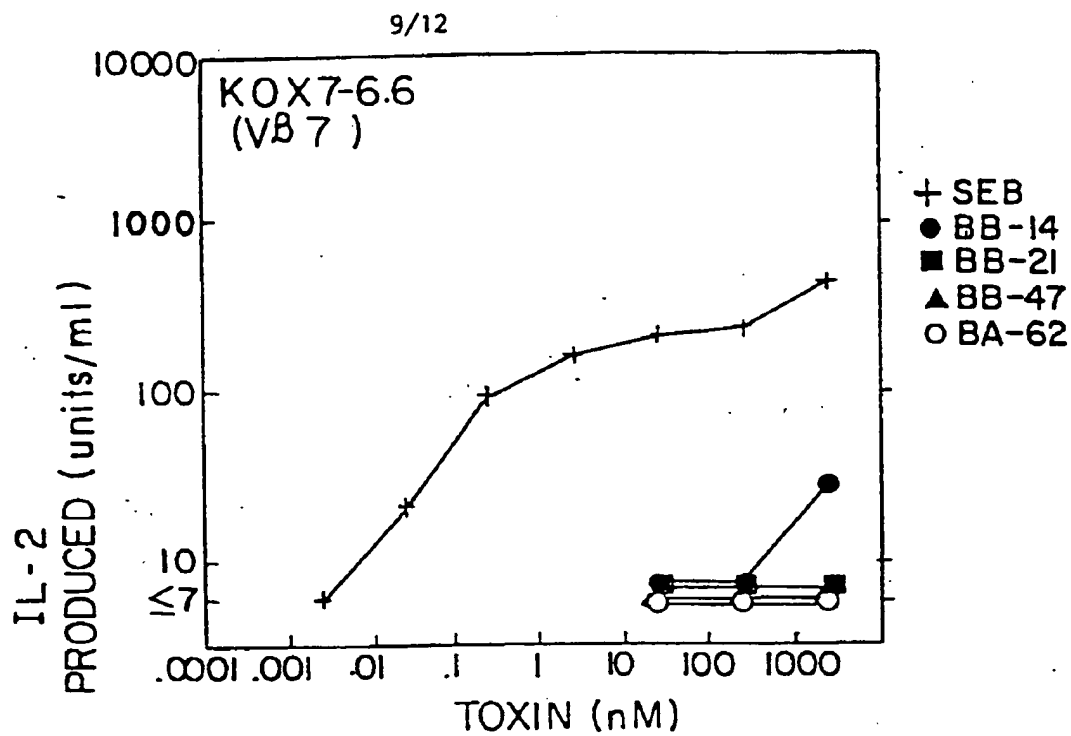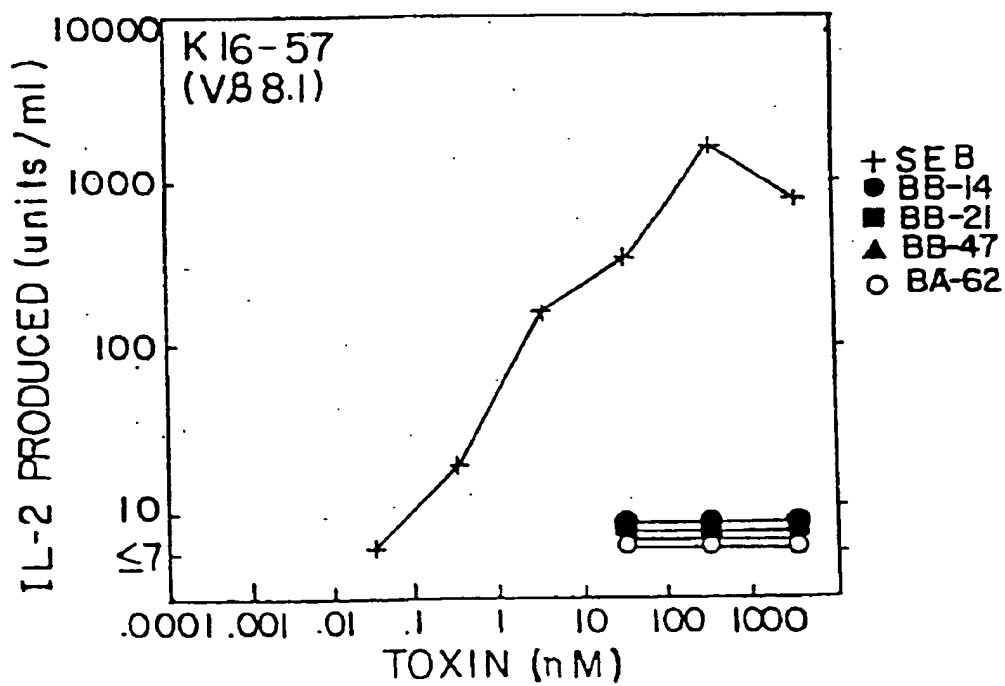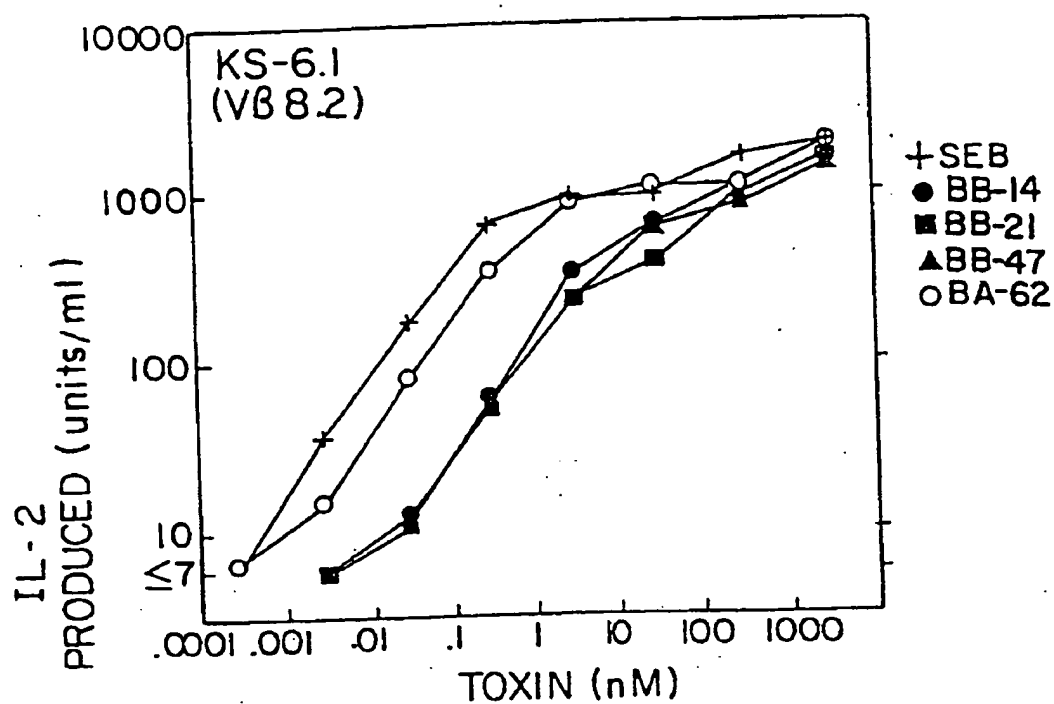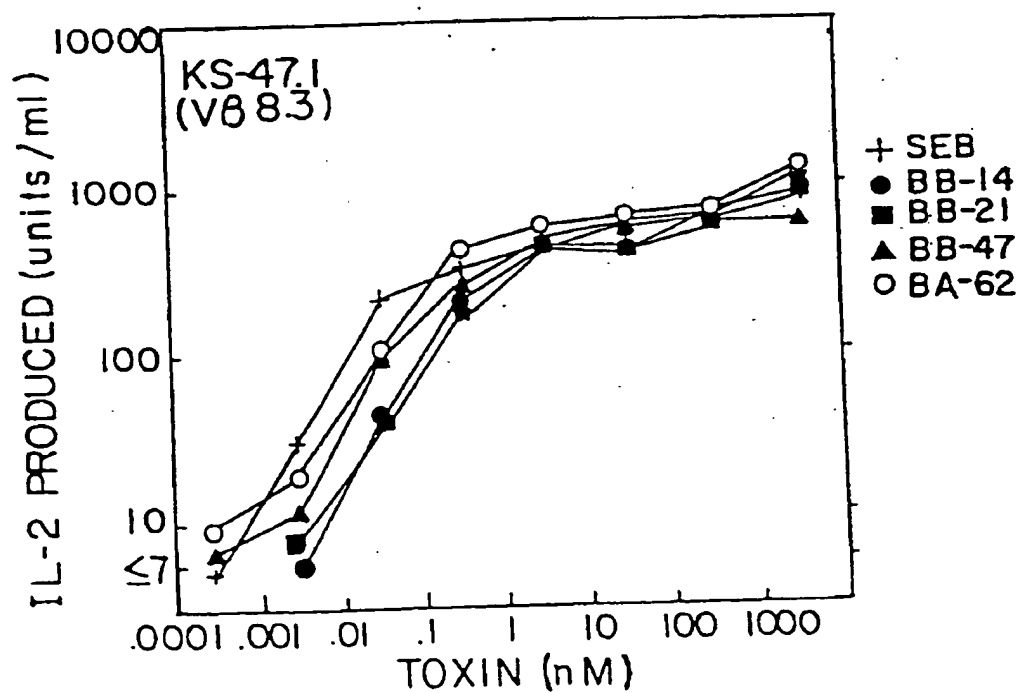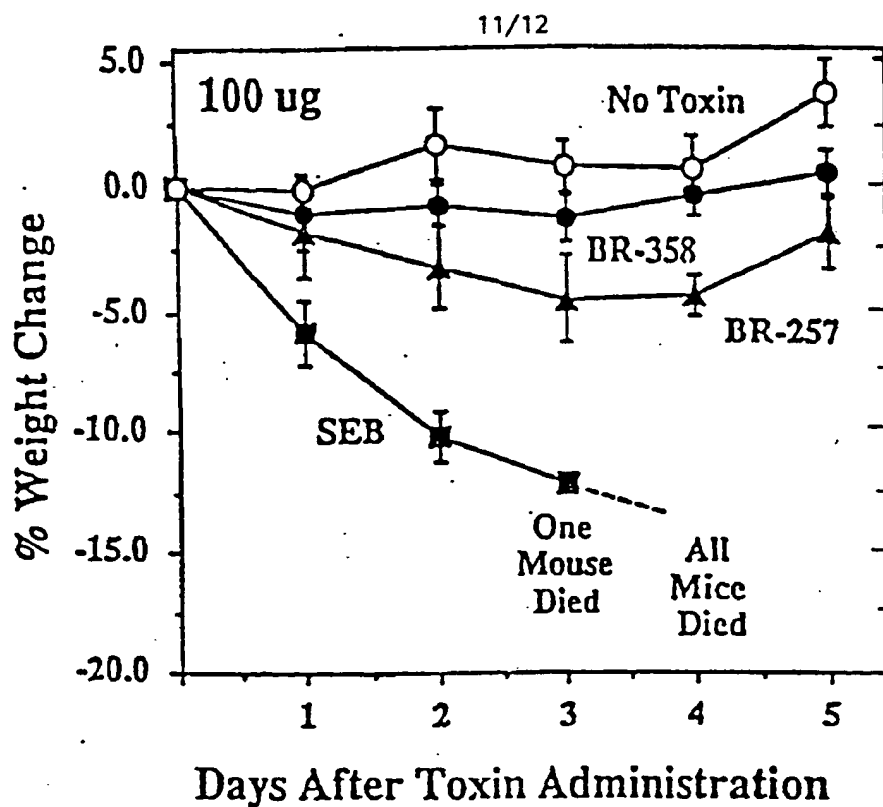

FIG.8A

FIG.8B

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FIGURE 9
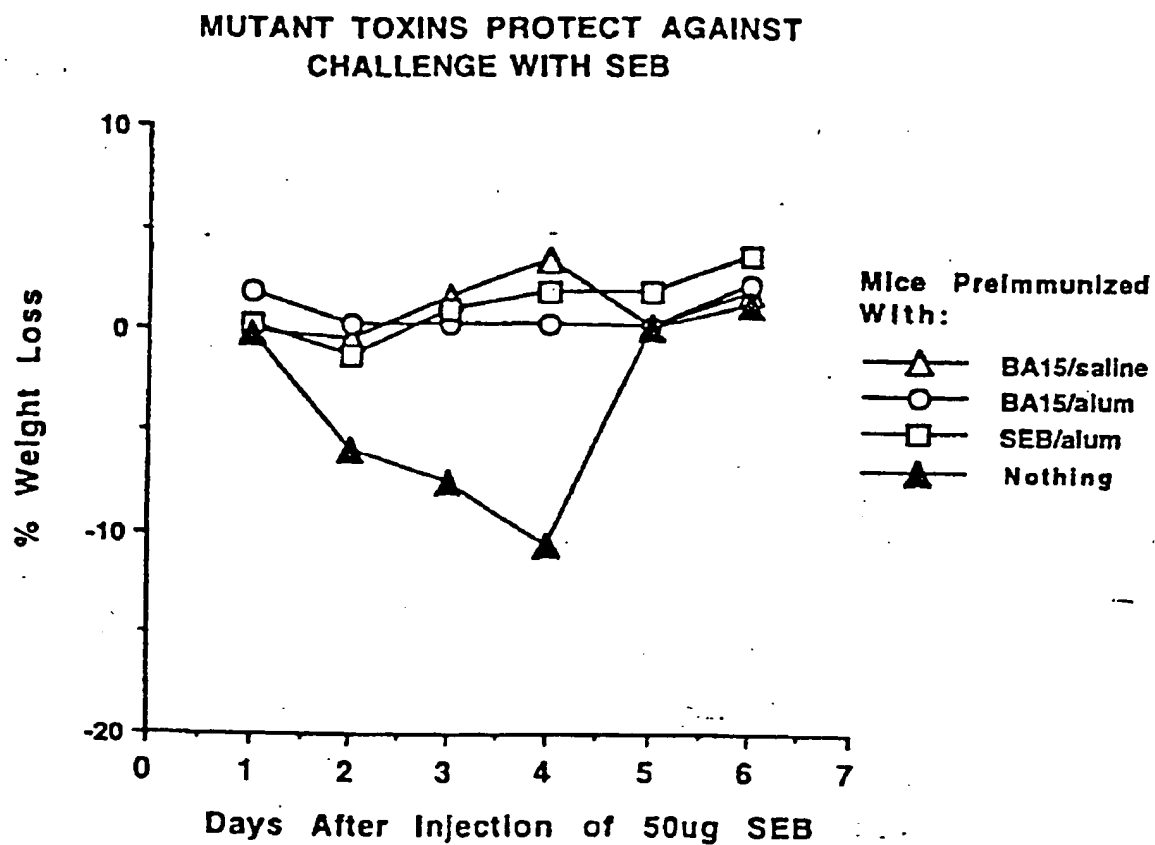
MUTANT TOXINS PROTECT AGAINST CHALLENGE WITH SEB

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/92; 435/7.1, 7.2, 7.33, 69.1, 69.3, 71.1, 172.3, 240.2, 320.1; 514/2; 530/350; 536/22.1, 23.1, 23.2, 23.4, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS
search terms: superantigen#, SEB, staphylo?, vbeta, enterotoxin, enterotoxin b

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Jour. Exp. Med., Volume 171, issued February 1990, P. Marrack et al., "The Toxicity of Staphylococcal Enterotoxin B in Mice is Mediated by T Cells", pages 455-464., see entire document.	1-9
Y	D. M. Glover, "Gene Cloning", published 1984, by Chapman and Hall (N.Y.), see pages 20-47.	1-9
Y	Jour. Bact., Volume 166, No. 1, issued April 1986, C. L. Jones et al, "Nucleotide Sequence of the Enterotoxin B Gene from Staphylococcus aureus", pages 29-33, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1993	04 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ROBERT A. WAX
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

International application No.
PCT/US93/00839

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A01 N 37/18, A61K 37/00, 39/02; C07K 3/00, 13/00, 15/00, 17/00; C12N 5/00, 15/00; C12P 21/04, 21/06; C12Q 1/00;

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/92; 435/7.1, 7.2, 7.33, 69.1, 69.3, 320.1; 514/2; 530/350; 536/22.1, 23.1, 23.2, 23.4, 23.7